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Effect of Auxin on Pectic Enzymes*

by Shizuko YODA**

依田静子**： オーキシンのペクチン質酵素に及ぼす影響*

Received October 23, 1957

It has been reported by the author and her collaborators¹⁾ that the stimulation of water uptake by auxin seems to be due to the plasticization of cell wall. Some other investigators^{2),3),4),5)} have concluded the same. And Kerr⁶⁾ considers that protopectin, which forms the continuous phase of the primary wall of the growing cell, may play a more important role in the plasticization of cell wall rather than cellulose, which constitutes a discontinuous phase. According to Ordin et al.⁷⁾, who studied the effect of indoleacetic acid (IAA) on the incorporation of C¹⁴-methyl-labeled methionine, more methyl C is incorporated in the pectic substance than in the cellulose fraction. Wilson and Skoog⁸⁾, using the section of tobacco pith, have confirmed that a marked change in the pectin substance precedes the elongation caused by IAA. Hence the pectic metabolism seems to be involved in the essential phase of growth induced by IAA. It has also been reported that, when the leaf and the stem of red kidney bean⁹⁾ are treated with 2, 4-D and the pith tissue of tobacco stem¹⁰⁾ with IAA, the pectin methylesterase (PME) activity of the homogenized tissues increases conspicuously.

If PME is activated significantly, the tensile strength of the primary cell wall will be reduced due to breakdown of pectin, and the wall will be stretched by the turgor of the cell.¹¹⁾ If auxin should also activate polygalacturonase (PG), which decomposes pectic acid to be produced from pectin by PME, the plastic stretching and the insertion of new material would further be remarkably facilitated.¹⁰⁾

The present paper reports some experiments designed to see the effect of auxin on the activities of PME and PG, in connection with effect of auxin on the water absorption, using the etiolated pea stem as material.

Material and Methods

Using commercial seeds of Alaska pea, seedlings were grown in the dark for 6 days at 25.5° C. The uppermost 5 mm of the 3rd internode, 3.0 to 3.5 cm in length, were decapitated. From the upper 1 cm zone of the remaining internode two 5 mm sections

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** Formerly Shizuko Kobayashi. Botanical Department, Faculty of Science, Kyoto University. Kyoto, Japan. 京都大学理学部植物学教室

were made. For the sake of comparison a material of low auxin sensitivity, the lowest 1 cm zone of the internode, cut into two 5 mm pieces, was used. A host of such section pieces was divided into batches of about 1 g fresh weight, to be used as the unit materials of the experiments.

As the aerobic treatment, stem pieces were floated on the surface of IAA solution and air was bubbled. As the anaerobic treatment, they were sunk at the bottom of the solution, 0.3–0.5 cm deep, intercellular air being previously removed by suction. The treatments lasted for 1.5 hours at 25.5° C. As the control, distilled water was used instead of IAA solution.

The water absorption was estimated as the percentage increase of the fresh weight in the 1.5 hour period of incubation.

Measurement of PME activity:—After weighing, the stem pieces were frozen and ground with dry ice. The powder obtained was suspended in 10% sodium chloride solution and used as the enzyme solution.¹²⁾ One percent solution of pectin ($\text{CH}_3\text{O}^*=9.45\%$ ¹²⁾) was used as the substrate.

The method used being Kertesz's method¹⁴⁾ modified slightly,^{12), 15)} the increase of carboxyl group was determined by sodium hydroxide titration. The PME activity was expressed in μM of methoxyl split off by the enzyme solution made from 1 g fresh weight of pea stems in 1 hour at $31 \pm 0.1^\circ\text{C}$. Fluctuations in duplicate measurements were within $\pm 2 \mu\text{M}$.

Measurement of PG activity:—The enzyme solution was prepared just as in the case of the PME assay. Pectic acid^{16), 17), 18), 19)} used as the substrate was ($\text{CH}_3\text{O}=0.59\%$ ²⁰⁾) prepared by demethylating commercial pectin by pectin methylesterase of tomato fruits.** The enzyme activity was expressed as the rate of increase of aldose as determined by Willstätter's method,²¹⁾ in μM per hour per g fresh weight of stem pieces. The activity was roughly the same at pH 4.0, 4.5 and 5.0, but a little lower at pH 6.0. Pectic acid coagulated at pH 3.5. Hence, the measurements were made at pH 4.5. Fluctuations in duplicate determinations were within $\pm 1.0 \mu\text{M}/\text{hour/g}$.

Results

A) Effect of IAA on pectin methylesterase activity.

The PME activity and the water absorption of the pea stem sections aerobically treated by various concentrations of IAA are presented in Fig. 1. It is clearly shown that the enzyme activity and the water uptake run almost in parallel with each other.

* Distilled ethanol was added to an equivolume of 10% solution of commercial apple pectin ($\text{CH}_3\text{O}=7.63\%$). The mixture was centrifuged, washed several times and dried *in vacuo*. Methoxyl radical was determined by Jansen's method.¹³⁾

** Homogenate of tomato fruits was centrifuged and extracted with 10% sodium chloride solution. Two percent solution of commercial pectin was treated at 40° C with this extract, adjusted to pH 6.0 with sodium hydroxide. After the hydrolysis was completed, the mixture was adjusted to pH 3.5 with hydrochloric acid, heated at 65° C. for 20 minutes and cooled. Pectic acid precipitating in the mixture was taken, dissolved in water and reprecipitated by hydrochloric acid. After the precipitate was rinsed with distilled alcohol until the chloric ion vanished, it was dried in air.

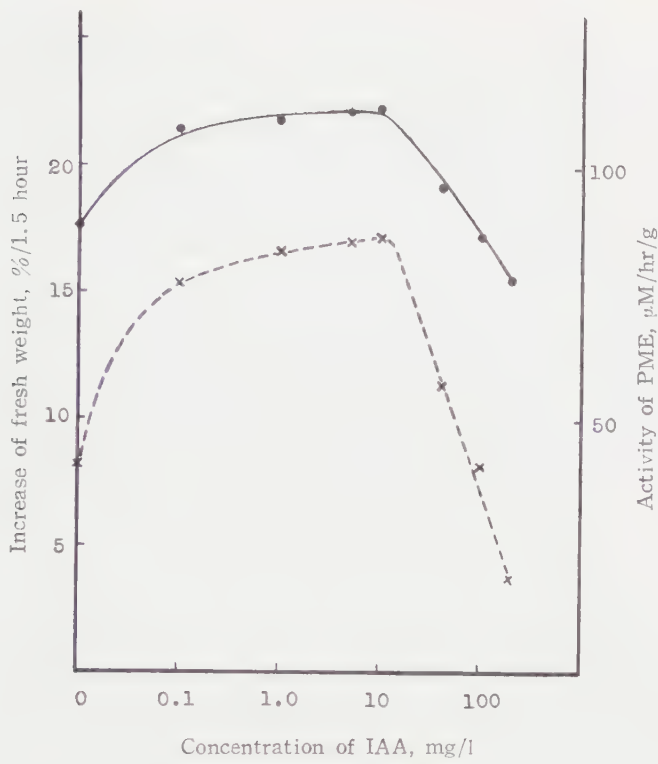


Fig. 1. Pectin methylesterase activity (—●—) and water absorption (—×—) of pea stem sections aerobically treated by various concentrations of IAA.

By the anaerobic treatment, on the other hand, neither the water uptake nor the PME activity was stimulated by auxin considerably. Typical results are described in Table 1. It is also shown in the table that under the aerobic conditions naphthaleneacetic acid stimulated both the PME activity and the water absorption less effectively than IAA did. and phenylacetic acid stimulated neither of them. The effect of auxin on the lowest portion of the internode is compared with that on the upper portion in Table 2. Both the water absorption and the PME activity were not increased by auxin in this part of low auxin sensitivity.

Table 1.

Effect of aerobic and anaerobic treatments by indoleacetic, naphthaleneacetic (NAA) and phenylacetic (PAA) acids on the pectin methylesterase activity and the water absorption of pea stem sections.

	PME activity (μ M/hr/g fresh weight)		Water absorption (100 × Δfr. wt./fr. wt./1.5hr)	
	Aerobic	Anaerobic	Aerobic	Anaerobic
Water control	88	82	8.5	7.7
IAA 10 mg/l	111	89	17.5	12.7
NAA 10 mg/l	99	—	12.4	—
PAA 10 mg/l	87	—	8.1	—

Table 2.

Effect of auxin on pectin methylesterase activity and water absorption of sections of the upper and the lower parts of the 3rd internode of pea stem.

	PME activity (μ M/hr/g fresh wt.)		Water absorption ($100 \times \Delta \text{fr. wt.} / \text{fr. wt.} / 1.5 \text{ hr}$)	
	Upper	Lower	Upper	Lower
Water control	88	62	8.5	5.5
IAA 10 mg/l	111	64	17.5	6.1

In order to see if auxin can affect the PME activity *in vitro*, various amounts of IAA were added to the enzyme solution prepared from the pea stems not treated by auxin. In Fig. 2, the auxin concentration is expressed in μ g of auxin per g fresh weight of tissue used for PME preparation. As seen in this figure, the PME activity was not changed by low concentrations of IAA, but was inhibited by high concentrations.

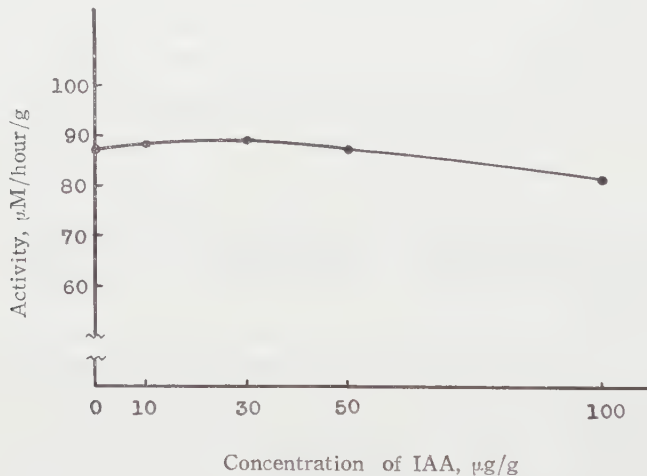


Fig. 2. Effect of IAA on the activity of pectin methylesterase in pea stem juice.

B) Effect of IAA on polygalacturonase activity.

The PG activity of the pea stem was very weak, and the assay fluctuation was comparatively large. However, it seemed likely that the PG activity was increased a little by the treatment of the tissue with 1 mg/l IAA, and was inhibited by the treatment with 10 mg/l the optimal concentration for the water absorption of the tissue in question (Table 3).

Table 3.

Polygalacturonase activity (μ M/hr/g fresh weight) of pea stem treated with various concentrations of IAA.

IAA concentration, mg/l				
0	1	5	10	20
5.2	5.9	5.4	3.3	1.6

Discussion

It was demonstrated that in a suitable concentration range IAA and NAA increased the PME activity of etiolated pea stem, as Bryan and Newcomb¹⁰⁾ had shown for the pith of tobacco. The PME activity and the water absorption of the tissue were changed by various treatments of the tissue, but they remained parallel to each other. These facts would imply that IAA plasticizes the cell wall by activating PME and the water absorption is accelerated by the decrease in the wall pressure. On the other hand, the activity of PG of the tissue was very low, and was not much increased by auxin. Moreover, the optimal IAA concentration for the PG activation differed from that for the water absorption. PG, therefore, is considered not to play a major part in the cell elongation.

In vitro, PME was not activated by IAA. Hence, the *in vivo* activation of PME by IAA must be associated with some change in the internal condition of the cell affected by IAA.

Summary

1. Measured were the activities of pectin methylesterase and polygalacturonase and the water uptake of etiolated pea stem which had been treated by auxin.

2. A remarkable parallelism was observed between the pectin methylesterase activity and the water absorption of the tissue, in respect to a) the concentration effect of IAA, b) the contrast between the aerobic and the anaerobic IAA treatments, and c) the weaker effects of naphthaleneacetic acid and phenylacetic acid as compared with IAA.

3. The activity of polygalacturonase was comparatively low, and the effect of IAA on it was not well paralleled by that on the water absorption.

4. The results appear favourable to the theory that the cell wall would become elongated the plasticization by pectin methylesterase activated by IAA.

Acknowledgements

The writer wishes to express her thanks to Prof. J. Ashida for his kind direction and helpful criticism throughout this work and also to Prof. I. Hatakeyama and Prof. G. Kuse for their valuable advices.

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On the Anthocyanin in the Blood-red Flower of *Tulipa Gesneriana* L.

Studies on the Physiology of Liliaceae II.

by Mannen SHIBATA* and (Miss) Emi SAKAI*

柴田 万年*・堺 恵美*: 血赤色花チューリップのアントシアニンについて
(ユリ科植物の生理学的研究 II.)

Received October 24, 1957

In 1916 R. Willstätter and E. K. Bolton for the first time identified qualitatively the flower pigment of a variety of *Tulipa Gesneriana* L. with cyanidin glucoside and carotene, and later G. M. Robinson and R. Robinson (1932) with the aid of an improved qualitative method of their own found that anthocyanin in the tulip flower exists mostly in the form of pelargonidin glucoside, occasionally accompanied with a small amount of cyanidin glucoside.

In 1953 M. Shibata succeeded in obtaining in crystalline state the pigment of a garden variety of tulip, "Queen of night", and in 1954 that of another garden variety, "Eclipse". The former was determined as rhamnoglucosidyl-delphinidin, and was tentatively named "Tulipanin" (M. Shibata, 1956).

The present study has been undertaken for the purpose of investigating the chemical properties of the flower pigment of "Eclipse". The bulbs of this variety were imported into Japan from Holland in 1950. The flower is blood-red and has a blue patch at the lower part of the perianth. Early in May, 1955, the material was collected at Mr. Yonebayashi's garden at Tonami city, Toyama Prefecture.

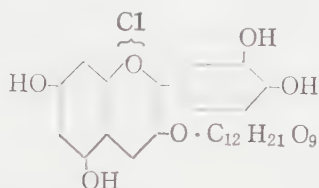
The process of isolation of the pigment is described in detail in the experimental part. The yield was 7.25 g, that is about 0.07 % of the fresh weight of the whole supply of perianths. The crystallized pigment was a glycoside which consisted of

* Biological Institute, Toyama University, Toyama, Japan. 富山大学文理学部生物学教室

cyanidin, glucose and rhamnose. So "Eclipse"-anthocyanin must be either one of "keracyanins" (R. Willstätter and E. H. Zollinger, 1916, K. Hayashi, 1941, K. Hayashi et al., 1954) or prunicyanin (R. Willstätter and E. H. Zollinger, 1916). "Eclipse"-anthocyanin closely resembles hitherto known keracyanins. It slightly differs, however, from them described in the literature in the number of molecules of water of crystallization and in solubility in dilute hydrochloric acid.

It melted undepressed on admixture with an authentic specimen of keracyanin. Its Rf-value was also identical with that of keracyanin.

From these results it is concluded that "Eclipse"-anthocyanin is identical with keracyanin (3-O-rhamnoglucosidylcyanidin) (Fig. 1).



"Eclipse"-anthocyanin (keracyanin)
 $C_{17}H_{31}O_{15}Cl \cdot 2H_2O$

Fig. 1.

Experimental

Isolation of "Eclipse"-anthocyanin as chloride.

About 10 kg of blood-red fresh perianths of "Eclipse"-flower, exclusive of pistils and stamens, were immediately immersed in 7 l of cold 2 % methanolic hydrochloric acid. After being allowed to stand for 20 hours the immersed perianths were pressed and filtered. Then the residual perianths were soaked in 2 l of methanol for 3 hours, pressed and filtered. This process was repeated once more. About 1 l of the combined dark red extracts obtained were mixed, under continuous stirring, with saturated solution of basic lead acetate, whereby white precipitate of lead chloride appeared first, and then bluish green precipitate of lead compound of anthocyanin was formed. The precipitate was filtered by suction, washed thoroughly with water and then with absolute ethanol and dried in a calcium chloride-containing desiccator, while the dirty purple filtrate, which no longer produced any precipitate with basic lead acetate, was discarded. The dried lead compound of anthocyanin was about 240 g in weight, inclusive of a considerable amount of lead chloride. It was then pulverized in a mortar, converted into chloride with 5 % methanolic hydrochloric acid and then filtered. The filtrate was concentrated to 1/2 volume *in vacuo* under 35°, chilled in a refrigerator and on addition of 3-5 volumes of ether precipitated anthocyanin chloride. After keeping in a refrigerator overnight, the supernatant liquor was decanted and the remaining hygroscopic amorphous anthocyanin was dissolved in a

minimum amount of absolute ethanol and filtered. The filtrate was added again with 2-3 volumes of ether and kept in a refrigerator overnight. The amorphous precipitate, which was no longer hygroscopic, was dissolved in a minute quantity of absolute ethanol and 1/2 volume of 20 % ethanolic hydrochloric acid was added to it. It soon became muddy, an indication that the crystallization of anthocyanin started. It was kept in a refrigerator for 2 days and then filtered. The yield was about 7.5 g. Then a small amount of cold 20 % ethanolic hydrochloric acid was added to the mother liquor and kept in a cold place for a few days, until a little more crystals of the red colouring principle were obtained. The total weight of the raw crystals was about 8.19 g. These crystals were dissolved in a minimum quantity of warm water and then filtered. An equal volume of cold 20 % ethanolic hydrochloric acid was added to the filtrate and the mixture was allowed to evaporate slowly, till the solution began to turn opaque and deposited crystals at the bottom of a crystallizing dish. The process was repeated again and about 7.25 g of pure crystals were obtained. The yield of the colouring principle therefore was 0.07 % of the fresh weight of the total perianths.

Anthocyanin chloride.

The "Eclipse"-anthocyanin, as given in Fig. 2, crystallized in red brown needles with a fine metallic luster. It melted at 177-179° (uncorr.), shrinking abruptly and turning black (decomp.). It was found to have 2 molecules of water of crystallization and to lose them *in vacuo* (2 mm Hg) at 105°, while the known keracyanins which it closely resembles are described to contain 6, 4, 3, or 2 1/2 molecules of water of crystallization. The solubility of "Eclipse"-anthocyanin increased with the decreasing concentration of hydrochloric

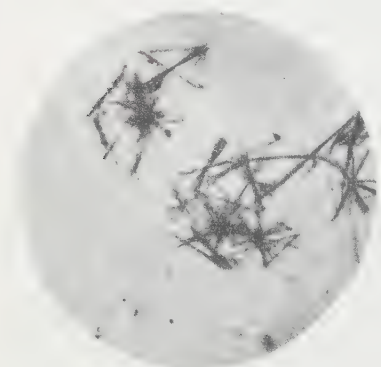


Fig. 2.

acid, whereas known keracyanins are reported to be readily soluble in 1 % hydrochloric acid and slightly soluble in either more or less than 1 % hydrochloric acid.

No depression of melting point was observed in the mixture of keracyanin (176°) and "Eclipse"-anthocyanin (177°).

Paper chromatography by the ascending one dimensional procedure was achieved at room temperature with acetic acid-36 % hydrochloric acid-water (3:1:8, v/v) and with use of the filter paper (Tôyô, No. 52, 40×40 cm). The paper chromatogram was shown in Fig. 3 and the R_f-values were as follows: keracyanin (K)=0.47, "Eclipse"-anthocyanin (E)=0.46 and the mixture of keracyanin and "Eclipse"-anthocyanin (E+K)=0.46. The fact that the keracyanin from *Canna generalis* (K. Hayashi et al., 1954) and "Eclipse"-anthocyanin gave the same R_f-value with the same developing solvent shows that they are the same compound.

The distribution number of "Eclipse"-anthocyanin, as measured after R. Will-

stätter and E. H. Zollinger(1916), was 6.7 and 7.1, while that of keracyanin from *Canna generalis* has been reported 6.7 (K. Hayashi et al., 1954).

The "Eclipse"-anthocyanin showed the absence of methoxyl group after Zeisel-Pregl method.

Anal. Calcd. for $C_{27}H_{31}O_{15}Cl$: C, 51.33; H, 4.92. Found: C, 51.08; H, 4.94.

Water of crystallization. Calcd. for $C_{27}H_{31}O_{15}Cl \cdot 2H_2O$: H_2O , 5.82. Found: H_2O , 5.41.

The absorption spectrum of "Eclipse"-anthocyanin was compared with that of the authentic specimen of keracyanin from *Canna generalis* with use of 5/10,000 mol of each colouring principle in 60 % ethanol containing 0.1 % hydrochloric acid.

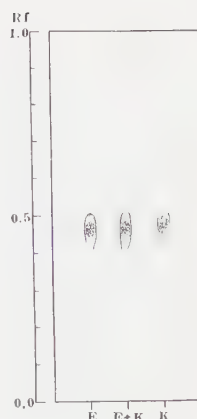


Fig. 3.

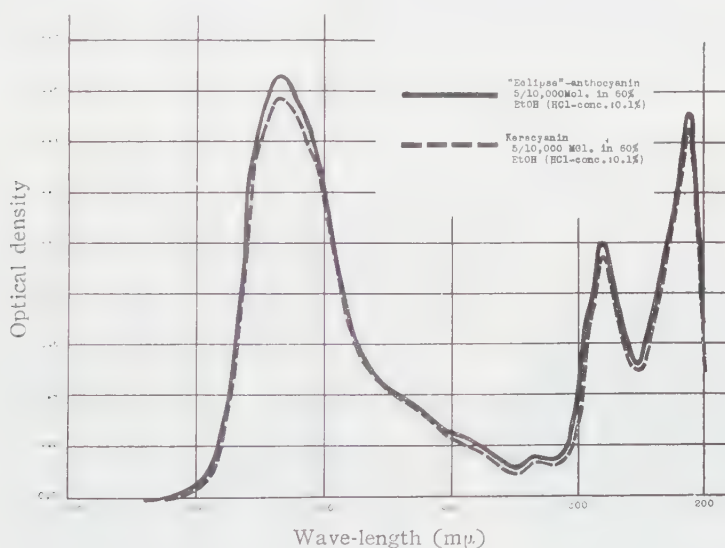


Fig. 4.

As seen from Fig. 4, the absorption curves coincide almost completely.

On the basis of these data, it seems reasonable to assume that keracyanin from *Canna generalis* and "Eclipse"-anthocyanin are the same compound.

Hydrolysis of anthocyanin chloride.

In 6 ml warm water were dissolved 284.0 mg of pure crystals of "Eclipse"-anthocyanin and the solution added with an equal volume of 36 % hydrochloric acid was boiled for 3 minutes. The solution, when allowed to stand for a day in a refrigerator, deposited a dark chocolate-brown crystalline substance (aglycone). The aglycone was collected, washed with 10 % hydrochloric acid and dried over sodium hydroxide in a desiccator. The aglycone obtained was 144.9 mg in weight, the yield being 51.02 % of the anthocyanin used.

The rose-coloured mother liquor was shaken thoroughly with iso-amyl alcohol

to remove the remaining aglycone. It was then shaken with ether to remove the small amount of iso-amyl alcohol and then neutralized with cold sodium hydroxide solution. The results of the qualitative determination by the orcinol and the phlorogrucinol tests and the diphenylamine test after Ihl-Peckmann-Jolles as well as by the paper chromatography indicated that the sugars in the solution were glucose and rhamnose.

In addition to the above mentioned tests, the mixed melting point determinations of the osazones of the sugars were carried out with the authentic specimens of rhamnosazone and glucosazone. The osazones of the sugars prepared by the usual method were treated with acetone: from the acetone soluble fraction crystalline yellow bushes (m. p. 179°) were obtained and from the acetone insoluble fraction fine yellow needles (m. p. 204°). On mixing the former with rhamnosazone (m. p. 180°) and the latter with glucosazone (m. p. 206°), no depression of melting point was observed.

It was concluded therefore that the sugars which combine with "Eclipse"-anthocyanidin are glucose and rhamnose.

Aglycone (cyanidin chloride)

The above mentioned crystals of the aglycone were recrystallized twice from 2% ethanolic hydrochloric acid in characteristic red brown needles. They presented the same appearance as described in detail in the report of R. Willstätter and E.

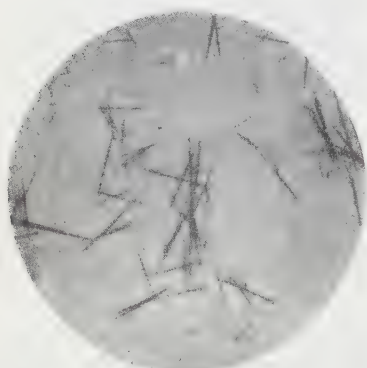


Fig. 5.

K. Bolton (see Fig. 5). They lost a molecule of water of crystallization *in vacuo* (2 mm Hg) at 105° and showed the following characteristics: they were easily soluble in ethanol and methanol; insoluble in water, dilute hydrochloric acid and concentrated sulphuric acid, either warm or cold; fairly soluble in 7% sulphuric acid and crystallized out as the sulphate of aglycone. The aglycone dissolved in hydrochloric acid was perfectly epiphasic with iso-amyl alcohol. The purple ethanolic solution of aglycone on addition of 2 volumes of water became colourless (pseudobase formation); on addition of ferric chloride solution, turned blue and then dirty green; turned reddish purple with 50% sodium acetate; blue with 50% sodium carbonate; when 20% lead acetate was added, formed a blue precipitate.

Anal. Calcd. for $C_{15}H_{11}O_6Cl$: C, 55.81; H, 3.44 Found; C, 56.17; H, 3.45.

Water of crystallization. Calcd. for $C_{15}H_{11}O_6Cl \cdot H_2O$: H_2O , 5.29. Found: H_2O , 4.77.

The properties and the results of the analyses coincide perfectly with those of cyanidin, indicating that the aglycone of "Eclipse"-anthocyanin is none other than cyanidin.

Summary

The anthocyanin in blood-red flower of "Eclipse", a garden variety of tulip (*Tulipa Gesneriana* L.) has been studied and it has been proved that it is none other than keracyanin (3-O-rhamnoglycosidylcyanidin $2H_2O$) by chemical analysis, absorption spectroscopy and paper chromatography.

We are indebted to Prof. K. Hayashi (Tokyo University of Education) for the valuable advice and the kind supply of the authentic specimen of keracyanin and to Mr. Yonebayashi for the donation of "Eclipse"-flower used in the experiment.

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Observational and Experimental Studies of Sensitive Plants IX. On the Canaliculated Intercellular Spaces of Primary Pulvinus*

Hideo TORIYAMA**

鳥山英雄*: ミモソウの葉の IX. 葉枕における溝状細胞間隙について

Received October 28, 1957

In one of the previous papers of this series of investigation, the author has confirmed the presence of canaliculated intercellular spaces in the motor tissue of *Mimosa pudica* (1). In the present report, the secretion of tannin substance into the canaliculated intercellular spaces were demonstrated in the fixed material. Here, the canaliculated spaces of the upper side and the lower side of primary pulvinus are chiefly dealt with.

Material and Method

The primary pulvinus of *Mimosa pudica* was employed for material. The plants were raised in pots exposed to the open air in the field. Anthocyan was found to

* Contribution No. 27 from the Biological Section, Tokyo Woman's Christian College.

** Biological Section, Tokyo Woman's Christian College, Tokyo, Japan. 東京女子大学生物学教室

be formed in the epidermal cells of the pulvinus, the petiole and the stem of these plants. For the observation of the motor tissue before receiving a stimulus, the plants were exposed to ether vapour for 15 to 20 minutes as usual, thus rendering the pulvinus of these plants incapable of responding to any stimuli.

Various fixatives, such as Kaiser's fluid, Bensley's fluid, and Carnoy's fluid were used. After soaking for two hours in Kaiser's fluid, the material was directly removed to 70 per cent alcohol with a few drops of iodine solution. The dehydration and infiltration of paraffin were performed by alcohol, butyl alcohol, butyl alcohol-paraffin and paraffin successively. The sections were cut at 10 micra and were stained with 0.5 per cent alcoholic toluidine blue solution, or with Mallory's triple stain. In animal micrology, it has been generally known that Mallory's connective tissue staining method differentiates the delicate changes in the protoplasm of animal tissue and its products according to the physiological conditions. By dint of this staining method it was found that the motor tissue got tinged differently after the stimulus was or was not given. The combination of Kaiser's solution-toluidine blue made it possible to demonstrate the tannin substance in the intercellular space by their greenish blue staining.

Observations and Experiments

As stated in the foregoing paragraph, with a purpose to know the presence of tannin substance in the motor tissue, the author tried first to demonstrated the tannin in the parenchyma by employing Kaiser's solution-toluidine blue method. In the motor tissue before the stimuli, the canaliculated intercellular spaces which contain the tannin substance appear very clearly due to its greenish blue stained by toluidine blue. It must be mentioned here that several special cells are found surrounding the canaliculated spaces. These cells have thin cytoplasm at their periphery, and do not contain tannin substance at all (Fig. 1. a, b). On the contrary, the surrounding parenchymatous cells are smaller than these special cells, and contain much tannin. The small intercellular spaces take the form of a canal system which contains tannin substance (Fig. 2. a, b). By fixation with Bensley's fluid, more particular details are observable. In the anaesthetized plants the tannin appears black with osmic acid as shown in Fig. 3. After the bending movement, almost the same figure appears also in the motor tissue. This phenomenon is observable also by other fixatives, e. g. by Carnoy's fluid. The tannin in these canaliculated spaces an appearance of the colloidal substance. It seems to the author that this figure suggests that the tannin solution has issued from the several parenchymatous cells into intercellular spaces. This supposition comes from the fact that the cells surrounding the vacuole do not contain the tannin substance as shown in Figs. 1, 2 and 4. There is no sign of change in these cells in both plants before and after receiving stimuli. By following the route of this canalicule filled with tannin it is realized that it leads to the hydathodes (Figs. 1, 4). The canalicule

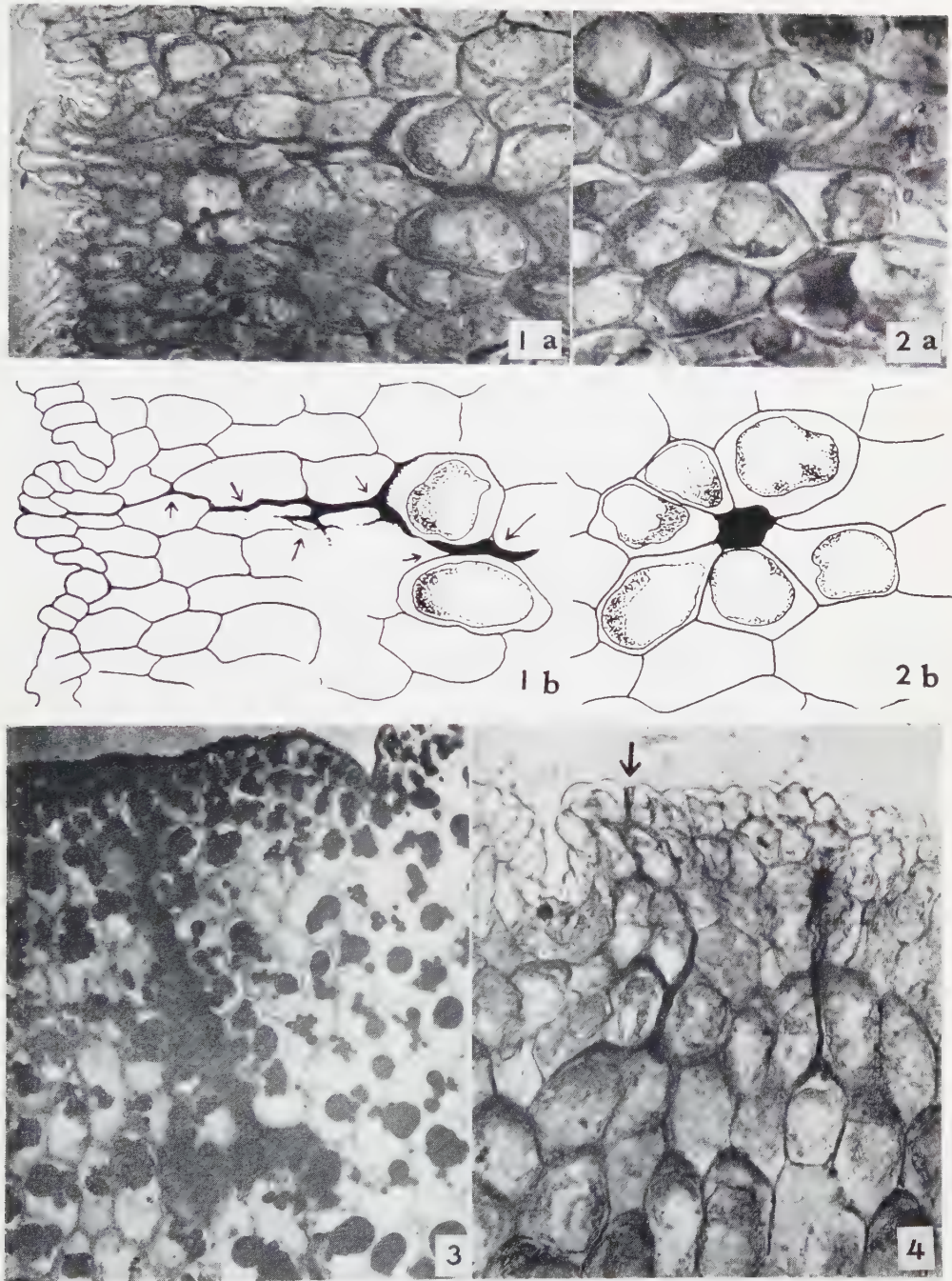


Fig. 1. a, b: Longitudinal sections of motor tissue. Tannin is issued into canaliculated inter-cellular space on the upper side of the primary pulvinus. Tannin is indicated by arrows. The tissue is fixed with Kaiser's fluid and stained with toluidine blue $\times 480$.

Fig. 2. a, b: Tannin is issued into small intercellular space, which is surrounded by several parenchymatous cells. The tissue is fixed with Kaiser's fluid and stained with toluidine blue. $\times 600$.

Fig. 3, 4: The upper side of the motor tissue. 3 is from the material treated with Bensley's fluid. $\times 320$. 4 is from the material treated with Kaiser's fluid. $\times 480$. Arrow indicates hydathode.

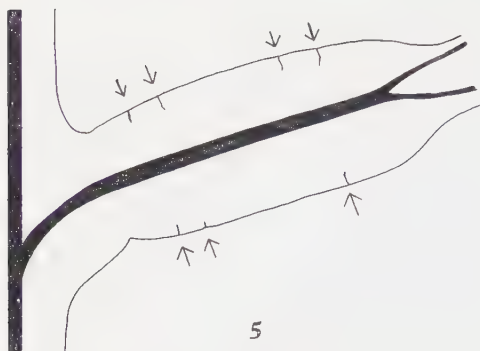


Fig. 5. Topographic demonstration to show the position and the depth of canaliculated intercellular spaces (shown by arrows).

lower region. Before proceeding further, it seems better for the reader's understanding to give a schematic figure which is summarized from the available data (Fig. 5). The canaliculated spaces are distributed most frequently in the periphery of the upper half and the lower half of the pulvinus.

Discussion

Kaiser's solution gave good results on the fixing of tannin substance in the motor tissue. The sections were at the same time stained with alcoholic toluidine blue solution which was very convenient for differentiating the tannin substance. On the other hand, the tannin solution in the intercellular spaces has strong affinity to the osmic acid. It is quite strange to the author that Dutt (2) said "The presence of tannin solution in the 'canaliculated' intercellular space of Toriyama* was not observed by me." But from the data of the author's experiment, it is concluded that the canaliculated intercellular space is one of the histological structures, and is not an artificial figure. These canaliculated spaces may be made to appear and not to appear, by different fixation reagents employed. Bensley's fixation is most suitable for the demonstration of the tannin in the intercellular spaces. The writer from his experiments knows that the tannin substance can be stained only by Kaiser's fluid or best by the Bensley's fluid, and is never stained vitally in the fresh material. These facts indicated that the juice which from the several parenchymatous cells is a dense liquid, and is not a mere water. At any rate it may be taken for granted that the permeability of these cells is increased due to some causes. And on the other hand, the secretion of tannin is by no means relevant to the bending movement of the primary pulvinus. But, at present, the function of canaliculated intercellular spaces remains a question. Yet, the author supposes that the tannin

* Toriyama, H. (1954)

becomes attenuated as it approaches to the hydathodes. Upon careful examination, though not clear in the photographs, these tannin substances are seen penetrating into a tiny pore, which is observable in a stained material.

On the surface of the upper half of primary pulvinus, these hydathodes are distributed 10 to 20 per mm². On the contrary, considerably fewer hydathodes are counted on the lower half. Nevertheless, the structure of the hydathodes observed from the surface view is always the same both in the upper and in the

substance has, with all probability, an ecological significance for the protection of some injurious insects. The evidence for this possibility must be the subject of future research.

The author wishes to express his cordial gratitude and hearty thanks to Prof. Sirô Tarao who has given him encouragement throughout this work.

Summary

The obtained data concerning the intercellular spaces in the pulvinus of *Mimosa pudica* may be summarized as follows:

1) Tannin substance in the motor tissue was fixed with Kaiser's and Bensley's fluids, and a 0.5 per cent alcoholic solution of toluidine blue was found to be a specific agent for staining the tannin.

2) In the motor tissue both before and after the stimuli, canaliculated intercellular spaces which contain the tannin appear very clearly by this technique.

3) The tannin from the several parenchymatous cells is thought to discharge into the canaliculated intercellular spaces.

4) From the data of these observations, it is concluded that the canaliculated spaces are by no means artificial figure.

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Cytogenetic and Cytoecological Studies on Some Japanese Species of *Rubus* I. Chromosomes

by Taro JINNO*

神野太郎*: 日本産ヤイチゴ属植物の細胞遺伝・生態学的研究 1. 染色体について

Received November 1, 1957

Introduction

Rubus is a large genus containing more than 200 species. More than a hundred species of them have been studied concerning their chromosome numbers (Chomistry 1927, Longley 1927, 1929, Longley and Darrow 1924, Crane and Darlington 1927, Crane 1929, Yarnell 1931, Crane and Laurence 1931, Datta 1932, Darrow and Longley 1933, and

* Biological Institute, Ehime University, Matsuyama, Japan. 愛媛大学教育学部生物学教室

Vaarama 1954). The results obtained by these investigators revealed a polyploid relation, i. e.; $n=7, \frac{21}{2}, 14, \frac{35}{2}, 21, \frac{45}{2}, 24, \frac{49}{2}$ and 28. Moreover, genom-analysis has been applied to a number of the species in this genus, and the phylogenetic relationships between some species and the relation between reproduction and autopolyploidy and allopolyploidy have been proved (Thomas 1940, and Crane 1940).

In Japan, according to Koidzumi (1913), there are 65 species, 8 subspecies, 10 varieties, 4 subvarieties, and 4 forms of *Rubus*. The present author collected some species of this genus from various localities of Japan, and he has carried out cytogenetic and cyto-ecological studies on them.

The writer wishes to express his cordial thanks to Prof. N. Shimotomai for his kind direction throughout this work. Hearty thanks are also due to Prof. Y. Horikawa for his kind direction concerning ecological problems. For the collection of materials used in this study, thanks are due to Dr. S. Okabe, Mr. H. Ochi, Mr. A. Moriya and Mr. H. Kubota.

Materials and Methods

The materials used in the present research are 24 species of Japanese *Rubus* (Table 1). Moreover, two garden varieties of *Rubus fruticosus* L. and a kind of Blackberry, were also studied.

The chromosomes of *Rubus* are not stained deep with acetocarmine and aceto-orceine, so that the other methods were used. The root-tips and anthers were fixed with Navashin's fluid and sections of them were made according to the paraffin-method, and they were stained with Heidenhain's iron-alum hematoxylin or with Newton's gentian violet. Moreover, the writer used the smear method, combining Feulgen's nucleal-reaction. The root-tips were pretreated for two hours with 0.003 M 8-oxyquinoline, and they were fixed with Carnoy's solution. Then, the materials were hydrolysed for seven minutes with 1 M HCl solution, and they were treated in Wermel's solution for 4-20 hours. The materials were placed on the slide glass and a drop of 45 % acetic acid was added to it and they were squashed with the cover glass.

Observation

The chromosome numbers were counted mostly in root-tip cells (Table 1). Of the 24 species studied 19 are diploid ($2n=14$), the others are tetraploid ($2n=28$), hexaploid ($2n=42$) and octoploid ($2n=56$). In *Rubus hakonensis* two forms have been found; the form collected in prov. Shimotsuke is hexaploid ($2n=42$), while the form found on Mt. Takanawa, prov. Iyo, is octoploid ($2n=56$). They differ from each other in some morphological characteristics; the octoploid has hairs on the stem, while that of the hexaploid is less hairy. The chromosome numbers which have been found in the Japanese *Rubus* are $2n=14, 28, 42$, and 56.

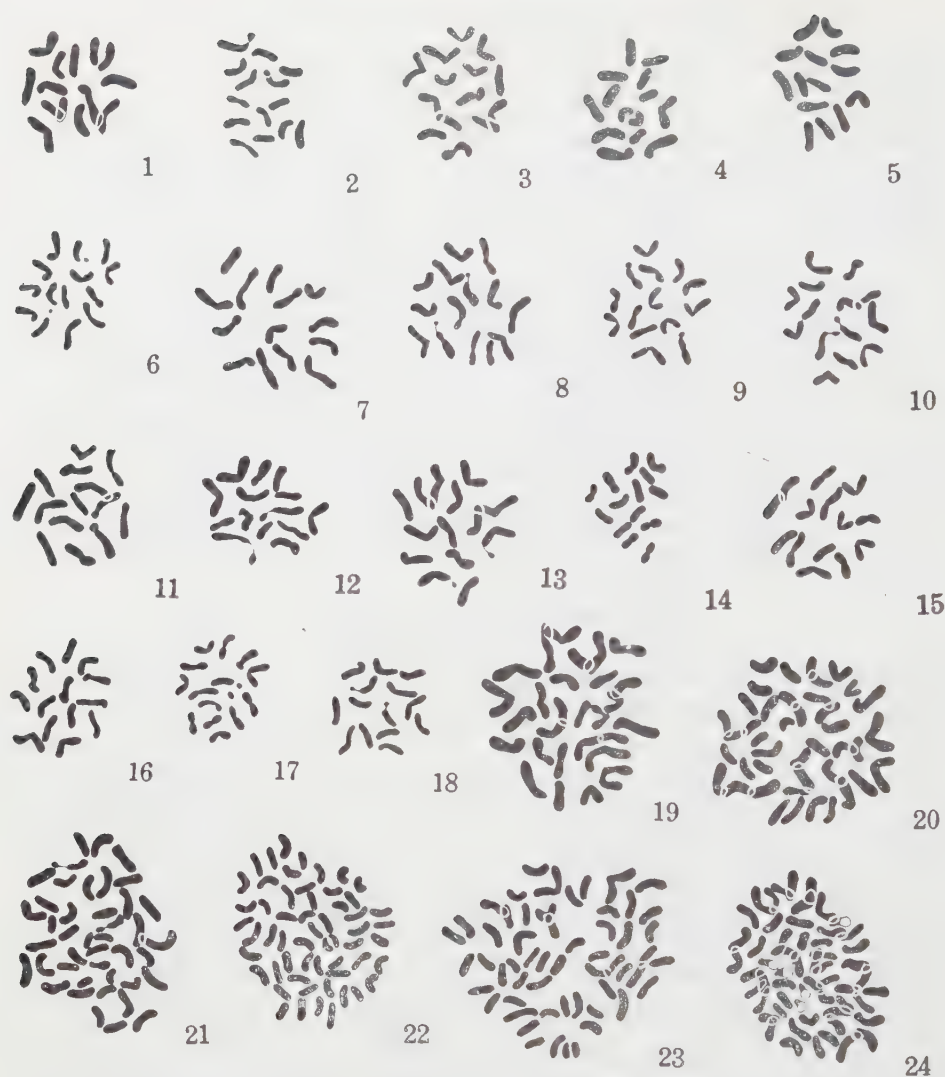
The chromosomes in the cell of the root-tip of *Rubus* are small in size, and

Table 1. Chromosome numbers of some *Rubus*.

Subgenera	Species name	Chromosome numbers 2n	Locality
CHAMAEBATUS	<i>R. pectinellus</i> Maxim.	42	Mt. Ishizuchi; prov. Iyo
CYLACTIS	<i>R. japonicus</i> Maxim.	14	Prov. Shimotsuke
ANOPLOBATUS	<i>R. ribisoides</i> Matsum.	14	Misaki; prov. Iyo
	<i>R. trifidus</i> Thunb.	14	Prov. Iyo
	<i>R. asper</i> Wallich	14	Kashima; prov. Iyo
	<i>R. coptophyllus</i> A. Gray	14	Manazuru; prov. Sagami
	<i>R. eustephanus</i> Focke var <i>coronarius</i> Koidz.	14	Prov. Iyo (cult.)
	<i>R. Fauriei</i> Lev. et Vnt.	14	Prov. Inaba
	<i>R. hirsutus</i> Thunb.	14	Prov. Iyo
	<i>R. illecebrosus</i> Focke	14	Mt. Ishizuchi; prov. Iyo
	<i>R. Koehneanus</i> Focke	14	Mt. Ishizuchi; prov. Iyo
	<i>R. microphyllus</i> L. var <i>incisus</i> Koidz.	14	Prov. Rikuchu
	<i>R. mesogaeus</i> Focke	14	Mt. Ishizuchi; prov. Iyo
	<i>R. parvifolius</i> L.	14	Prov. Iyo
	<i>R. peltatus</i> Maxim.	14	Mt. Ishizuchi; prov. Iyo
	<i>R. palmatoides</i> O. Kuntze.	14	Mt. Takanawa; prov. Iyo
	<i>R. phoenicolasius</i> Maxim.	14	Mt. Takanawa; prov. Iyo
IDAEOBATUS	<i>R. pseudo-Acer</i> Makino	14	Mt. Ishizuchi; prov. Iyo
	<i>R. Wrightii</i> A. Gray	14	Mt. Takanawa; prov. Iyo
	<i>R. Yabei</i> Lev. et Vnt.	14	Mt. Ishizuchi; prov. Iyo
	<i>R. Sieboldi</i> Blume	28	Coast of prov. Iyo
	<i>R. hakonensis</i> Franch. et Sav.	42	Prov. Shimotsuke
		56	Mt. Takanawa; prov. Iyo
	<i>R. Buergeri</i> Miq.	56	Mt. Takanawa; prov. Iyo
	<i>R. pseudo-Sieboldii</i> Makino	56	Mt. Tsubogami; prov. Iyo
MALACHOBATUS			

even the largest ones are about 2-3 μ long. These chromosomes, when fixed with Navashin's solution without pretreatment and stained with Heidenhain's iron-alum hematoxylin solution, are V-, J-, or I-shaped. In some diploid species there is, among 14 chromosomes, a pair with satellites.

When the following process of the smear method is employed the constriction and the satellite of the chromosome are observed more clearly than when the method mentioned above is used; that is, chromosomes are pretreated to contracted and stained by the Feulgen method. The chromosome morphology of the five species which are observed in this way are shown in Figs. 25-29. The largest pair of chromosomes in each of the five species has constrictions at median, but the remaining chromosomes have constrictions at median, submedian or subterminal. And the frequencies of occurrence of these three kinds of chromosomes in a genom differ from species to species. Of the five species four have a pair of medium-sized



Figs. 1-24. Somatic chromosomes in 24 species of *Rubus*. 1) *R. parvifolius*. 2) *R. ribisoideus*. 3) *R. eustephanus* var. *coronarius*. 4) *R. hirsutus*. 5) *R. phoenicolasius*. 6) *R. palmatoides*. 7) *R. trifidus*. 8) *R. Fauriei*. 9) *R. japonicus*. 10) *R. Koehneanus*. 11) *R. mesogaeus*. 12) *R. microphyllus*, var. *incisus*. 13) *R. Yabei*. 14) *R. Wrightii*. 15) *R. illecebrosus*. 16) *R. peltatus*. 17) *R. asper*. 18) *R. coptophyllus*. 19) *R. Sieboldi*. 20) *R. pectinellus*. 21) *R. hakonensis*. 22) *R. hakonensis*. 23) *R. pseudo-Sieboldii*. 24) *R. Buergeri*.
 ×2600

chromosomes with satellites. The satellite of *R. hirsutus* is smaller than those of the other species. *R. coptophyllus* has a pair of large chromosomes with secondary constrictions.

The conjugations of chromosomes of meiosis in P.M.Cs. of these three natural polyploids were observed. In *R. Sieboldi* ($2n=28$) 14 bivalents are formed at the first metaphase. In *R. Buergeri* ($2n=56$) there occur mostly 28 bivalents. But

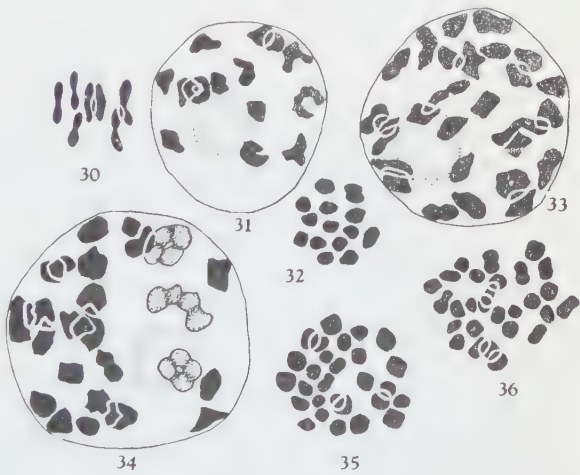
sometimes besides bivalents a few tetravalents are also formed. The form of the tetravalent is a chain or a ring. In *R. pseudo-Sieboldii* 28 bivalents are observed at the first metaphase.

In *R. fruticosus* L. var. (cultivate, $2n=28$), the common configurations of chromosomes in meiosis are $13_{II}+2_I$ or 14_{II} , and the former occurs much more frequently (about 70 %) than the latter. The univalents are often left in the cytoplasm and form micronuclei. When tetrads are formed, there often can be found small cells between larger ones. The pollen grains are of various sizes. And those which seem to be normal are oblong with the larger diameter of about 40μ , and the smaller ones, which amount to 30 % of all the pollen grains, are irregular in shape and scarcely germinate. The differences in the sizes of the ovule, the ovary and the drupes in this species are thought to be connected with the above-mentioned abnormality in meiosis.

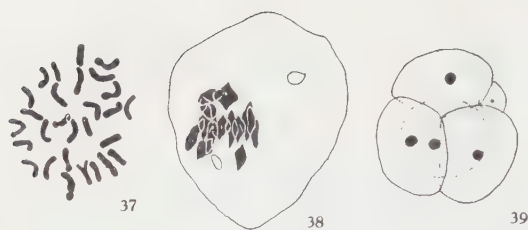
A kind of Blackberry which was cultivated in the garden was cytologically studied. It is a heptaploid ($2n=49$). The chromosome configurations at the first metaphase are $21_{II}+7_I$. The bivalents arrange them-



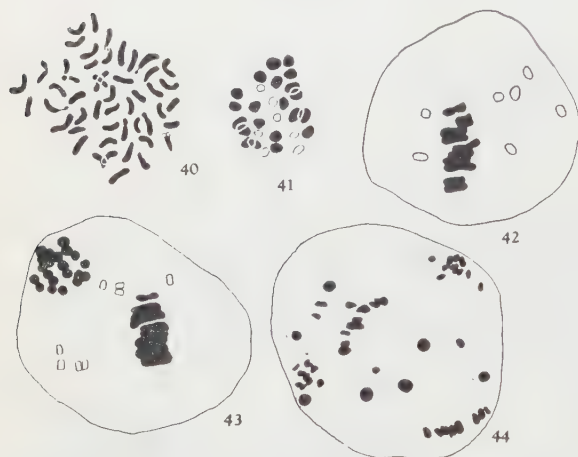
Figs. 25-29. Somatic chromosomes of 5 species of *Rubus*. 25) *R. trifidus*. 26) *R. parvifolius*. 27) *R. coptophyllus*. 28) *R. hirsutus* 29) *R. microphyllus* var. *incisus*. $\times 3000$.



Figs. 30-36. Meiosis of P.M.C. of *R. palmatoides* (diploid), *R. Sieboldi* (tetraploid), *R. Buergeri* (octoploid), and *R. pseudo-Sieboldii* (octoploid). 30: Side view of IM of *R. palmatoides*, 7_{II} . 31: Diakinesis of *R. Sieboldi*, 14_{II} . 32: Polar view of IM of *R. Sieboldi*. 14_{II} . 32: Diakinesis of *R. pseudo-Sieboldii*, 28_{II} . 34: Diakinesis of *R. Buergeri*, $3_{IV}+22_{II}$. 35: Polar view of IM of *R. pseudo-Sieboldii*, 28_{II} . 36: Polar view of IM of *R. Buergeri*, 28_{II} . $\times 2500$.



Figs. 37-39. Meiosis and somatic chromosome in *R. fruticosus* var. 37) Chromosome in root-tip. 38) First metaphase. 39) Pollen-tetrad having a micro-cell. 37 $\times 1900$, 38 $\times 2100$, 39 $\times 1500$.



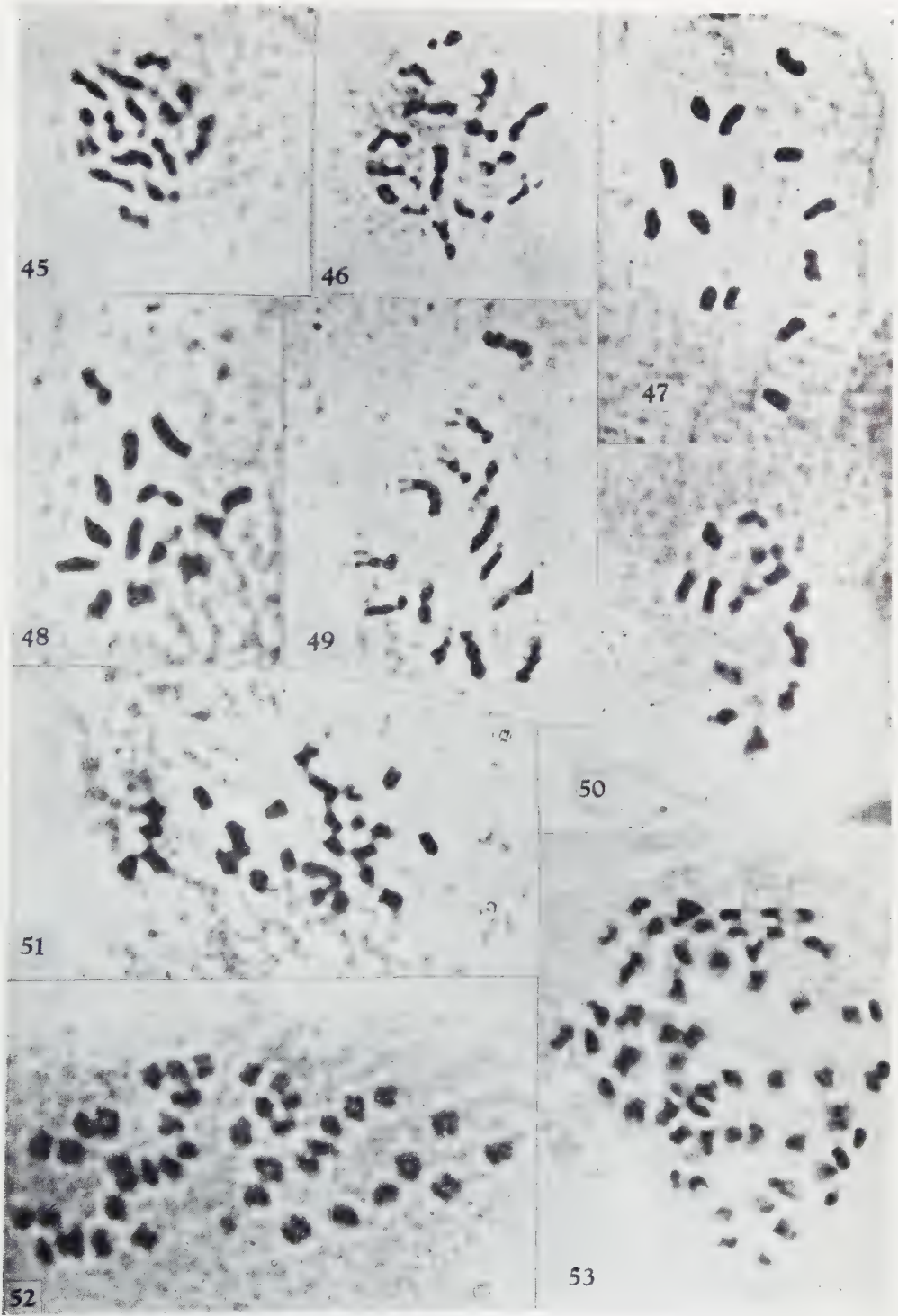
Figs. 40-44. Meiosis and somatic chromosomes in *R. sp.* (Blackberry). 40) Chromosomes in root-tip cell. 41, 42) First metaphase. 43.) Second metaphase. 44) Second anaphase with micro-nuclei. 40 $\times 1900$, 41-44 $\times 2100$.

selves in the equatorial plate and the univalents are scattered outside of it. At the anaphase of the second division, several micronuclei are very often formed in the cytoplasm, which give rise to abortive pollen grains.

Discussion

This genus *Rubus* is divided into several subgenera; e.g. *Chamaebatus*, *Cylactis*, *Anoplobatus*, *Idaeobatus*, *Malachobatus* etc. All the species belonging to subgenera *Idaeobatus* and *Anoplobatus* are, so far as the investigation has been made, diploids. Those which come under Subgenera *Chamaebatus* and *Malachobatus* are all polyploids, and the latter comprises tetraploids, hexaploids and octoploids. It may be pointed out that speciation has occurred more evidently in these two subgenera than in the others.

R. pseudo-Sieboldii is one of the natural species occurring in the south-west parts of Honshu and Shikoku. Makino (1926) reports that this plant may be regarded as a hybrid between *R. Sieboldii* and *R. Buergeri*. The comparative morphological observations on these three plants have shown that *R. pseudo-Sieboldii* is intermediate between *R. Sieboldii* and *R. Buergeri* in some outer characteristics: the lamina, the stipule and the form of the stem. The chromosome number of *R. Sieboldii* is $2n=28$, while that of *R. Buergeri* is $2n=56$. The F_1 between the two species should have $2n=42$ chromosomes. But *R. pseudo-Sieboldii* has $2n=56$ chromosomes. And they do not bloom at the same time. From these facts it follows that *R. pseudo-Sieboldii* is not the F_1 between both species. Therefore, this species is thought to have been produced at a certain time in the past by the ancestral hybrids which are supposed to have had some parts of the genomes of these two species.



Figs. 45-53. Photomicrographs of somatic chromosomes (smear method). 45) *R. ribisoideus*. 46, 47) *R. trifidus*. 48) *R. coptophyllum* 49) *R. parvifolius*. 50) *R. hirsutus*. 51) *R. Sieboldi* 52) *R. pectinellus*. 53) *R. Buergeri*. $\times 3250$

Makino (1948) reports that from the taxonomical viewpoint *R. palmatoides* and *R. coptophyllus* are closely related. As the result of comparative observations of these chromosomes, the writer has found that the chromosomes of these two species are similar in shape. Therefore, the writer believes, as Makino reports, that systematically these species may be closely related to each other.

Summary

1. The present writer has studied cytologically 23 species of *Rubus* which were collected from various localities of Japan and 3 garden *Rubus*. Their chromosome numbers were confirmed to be $2n=14, 28, 42, 49$ and 56 , showing a polyploid series with a basic number $n=7$.

2. As far as the writer's investigation has been made, subgenera *Idaeobatus* and *Anoplobatus* include only diploids, while subgenera *Chamaeobatus* and *Mala-chobatus* consist of polyploid species.

3. In meiosis of *Rubus pseudo-Sieboldii*, which is a natural octoploid of hybrid origin, only bivalents or bivalents with a few tetravalents are formed.

4. In the garden variety of *R. fruticosus* ($2n=28$), the most frequent configurations of chromosomes are $13_{II}+2_I$. A kind of Blackberry was confirmed to be heptaploid ($2n=49$). In meiosis of this plant the configurations of chromosomes are $21_{II}+7_I$. The pollen grains of these two garden plants consist of normal and abortive ones.

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Developmental Mechanics of Fucaceous Algae VIII. Blister Formation in Some Furoid Eggs

by Singo NAKAZAWA*

中沢信年*: フーケス科藻類の発生力学 VIII. 卵におけるブリスターの形成

Received November 7, 1957

It has been revealed that the cell wall of furoid eggs is progressively hardened after fertilization^{1),5)}. This phenomenon plays an important rôle in the polarity determination. That is, the polarity of these algae begins to appear with elongation of the egg form, the membrane hardening proceeds on this occasion and the elongated egg form becomes stabilized by the rigid cell wall, which leads the next stage to the polarity determination. This has been observed in *Coccophora*^{4),5)}, *Fucus*^{3),6)}, and in *Sargassum*^{2),5)}. Here, the present writer reports his experiments on blister formations in some furoid eggs treated with zinc chloride solution to reveal the rôle of membrane hardening in the determination.

The experiments were carried out 1) on *Coccophora Langsdorffii* in April of 1957 at the Marine Biological Station of Asamushi, Aomori Prefecture, 2) on *Fucus evanescens* in May of 1956 at the Institute of Algological Research, Muroran, Hokkaido, and 3) on *Sargassum confusum* in May of 1956 at the Marine Biological Station of Asamushi. In each of these algae, eggs were liberated and fertilized in filtered sea water contained in glass vessels. After the fertilization, eggs were placed on slide glass, there some drops of zinc chloride solutions of various densities were added to, and these were observed.

Not a remarkable change was observed at any stage of the development with zinc chloride at a density lower than 15 per cent. But clear knobby blisters (Fig. 1b)

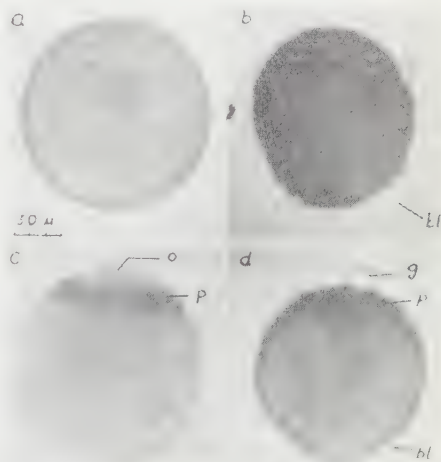


Fig. 1. *Coccophora* eggs. a) Normal egg, b) blister formation in a normal egg, c) entrifuged egg, d) blister formation in a centrifuged egg. bl, blister; g, giant blister; o, oil layer at the cetripetal end; p, plastid layer.

* Biology Department, Yamagata University, Yamagata, Japan. 山形大学生物学教室

Table 1

Density of zinc chloride and time required for the blister formation in *Coccophora* eggs just after fertilization.

Density in per cent	Blister formation	Time required in seconds
60	+	30
50	+	50
25	+	360
15	±	
10	—	
5	—	
0	—	

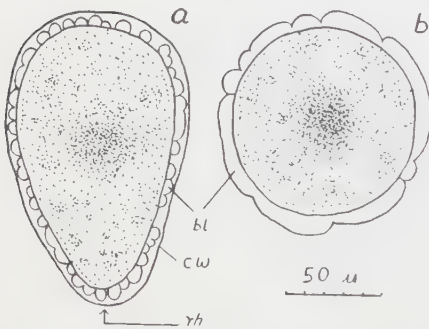


Fig. 2. *Coccophora* eggs. a) Blisters formed in an egg after the polarity determination, b) blisters united to form a layer surrounding the egg. *bl*, blister; *cw*, cell wall; *rh*, rhizoid pole.

were raised over the egg surface at a density higher than 25 per cent (Table 1). The blisters appear more remarkably at a younger stage than after the polarity determination, i.e. after the egg transformation. The blisters increase in number in time. The time required for their first appearance differs according to the density of zinc chloride solution. The higher the density, the shorter the time so far as experimented. The part where blisters begin to appear is not definite, but they are raised in random parts on the egg surface, then more blisters come out successively, and sometime they unite together to form a colourless layer, which surrounds the egg (Fig. 2b). After two or three hours, the blisters begin to dissolve naturally and at last disappear. The blister can be stained vitally with brilliant green or with aurantia, but cannot be stained with Brasilin, eosin, Janus green B, neutral red, Nile blue, or with Sudan III. It is not dissolved with 50 or 70 per cent alcohol, but can be dissolved with

sea water or more promptly with distilled water. After the morphological polarity was determined, viz. the egg form was elongated, plasmolysis occurs with addition of zinc chloride, that is the egg protoplasm is separated from the rigid cell wall which has been hardened parallel with the transformation of the egg form. As a result, the blisters are formed in the space between the plasm and the cell wall (Fig. 2a). At two-, three-, or more-cell stage, in *Coccophora* and *Sargassum*, blister formation does not or does occur not so remarkably as at the younger stage. In *Fucus*, it still takes place very remarkably at two-cell stage, but it gradually fails to occur as the stage proceeds.

When *Coccophora* or *Sargassum* eggs are centrifuged before or just after the fertilization at 1500 times gravity for 30 minutes, clear stratification is derived, so that oil drops are gathered at the centripetal end as was reported in the writer's previous papers^{2),5)}. In these centrifuged eggs the blister formation occurs in a different manner. A giant yellow blister appears at the centripetal end (Fig. 1d) in addition to the colourless normal blisters which appear in other parts. The

giant blister seems to be a protrusion of the oil gathered at the centripetal end. Actually, with appearance of the giant blister, the volume of the oil layer becomes to be much diminished, so that the centripetal end is a little hollowed inwards. Besides, the giant blister can be stained with Sudan III in pink colour different from the normal blister.

Judging from that the normal blisters formed in eggs which were not centrifuged are colourless and cannot be stained with Sudan III, they are not of lipid nature, they must be composed of hyaloplasm because of their property of being dissolved with water but not dissolved with alcohol. That is to say, it seems that the blisters are drops of hyaline plasm extruded from the egg cell through a number of considerably small holes of the plasma membrane brought about by addition of zinc chloride. This can be verified by that the giant blister, the extruded oil drop, appears always at the centripetal end where the oil was gathered by centrifugation, while the normal hyaline blisters are formed not at the centripetal end but in the other part on the surface of the same centrifuged egg. The presumed holes, through which the hyaline plasm extrudes to form blisters, seem to be very small considering that the blister does not contain a plastid. The cell wall at a young stage appears to be very fragile as it is easily penetrated by the extruding hyaline plasm, the blisters. At a later stage, after the morphological polarity was determined, the plasma membrane is separated from the cell wall with zinc chloride and the blisters are formed in the space intervening between the plasma membrane and the cell wall. This phenomenon indicates the occurrence of progressive hardening of the cell wall with the polarity determination, which results in an irreversible change of the egg form pointed towards the rhizoid pole.

Summary

When eggs of *Coccolophora Langsdorfii*, *Fucus evanescens*, or *Sargassum confusum* are treated with zinc chloride solution at a density higher than 25 per cent, clear blisters are formed over the egg surface. After the morphological polarity was determined, plasmolysis occurs with zinc chloride so that the protoplasm is separated from the cell wall and the blisters appear in the space between the plasm and the cell wall. This indicates that the cell wall has been hardened parallel with the polarity determination. The blisters seem to be composed of the hyaline plasm extruded through small holes of the plasma membrane brought about by treatment with zinc chloride.

The writer expresses his cordial thanks to Prof. I. Motomura, Tôhoku University and Prof. B. Wada, University of Tokyo for their valuable suggestions in the blister formation.

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帽菌類の diploidisation における核の行動 II. 両和合性組合わせの場合*

木 村 勘 二**

Katsuji KIMURA**: Nuclear Behaviour in the Diploidisation of the Hymenomycetous Fungi II. Doubly Legitimate Combinations*

昭和 32 年 8 月 26 日受付

及川 (1939) は *Galera tenera* (四極性) の 2 系統を用いて、 $AB \times (A'B' + a'b')$ のような型の両和合性組合わせによる diploidisation の実験を行った。そして diploidisation を起した AB 菌叢の周縁部並びに内部の数ヶ所から菌糸をとって、その核の構成を分析したところ、ある一つの組合わせで周縁部に現われた複相菌糸の一部は $AB + A'B'$ であり、他の一部は $AB + a'b'$ であったような結果を得たことから、接種した両和合性複相菌糸の $A'B'$, $a'b'$ の 2 核は単相大菌叢の中を移行するものであらうと述べている。しかし、このような結果は稀れて、他の組合わせの全部は大菌叢の周縁部、内部をとわず、どの部分も等しく $AB + A'B'$ か又は $AB + a'b'$ のどちらか一方だけであったことから、 $A'B'$, $a'b'$ 2 核のどちらか一方が他方よりも早く核分裂しながら AB 菌叢の中を進むものであらうと推論している。

Papazian (1950) は *Schizophyllum commune* (四極性) の 2 系統を用いて、同じく $AB \times (A'B' + a'b')$ のような実験を行い、大菌叢の周縁部に現われた複相菌糸の 2 核を独特の方法で分析した。そして、ある組合わせでは、何度行っても出現複相菌糸の 2 核は常に $AB + A'B'$ であって $AB + a'b'$ は出なかったというような結果の外に、

別の組合わせでは $AB + A'b'$ あって、接種した複相菌糸の 2 核が染色体を交換して新核を形成したと思われるような結果、或いは又 $A'B' + a'b'$ であって接種した複相菌糸からの 2 核移行を証明するような結果をも得ている。Quintanilha (1938) も *Coprinus fimetarius* (四極性) の 2 系統を組合わして同様な実験を行い、上述のような 2 核移行を示す実験例を一つ得ている。

著者 (1957b) は *Coprinus macrorrhizus* f. *microsporus* の数系統を供試して $AB \times (ab + A'B')$ のような型の両和合性の組合わせを行い、85 の組合わせの中、出現複相菌糸が $ab + A'B'$ であったような 2 核移行を示す結果を 9 例得た。

Buller (1941) は及川や Quintanilha の結果から推して、他の不和合性や和合性の組合わせはさておき、少なくとも両和合性組合わせにおいては接種した複相菌糸の 2 核は移行するものであらうと述べているが、著者等の結果から見ても、このことは間違いないと考えられる。しかし、複相菌糸を接種してから 2 核が移行し、周縁部に複相菌糸を作るまでの経過については僅かに及川の移行 2 核の遅延についての考察があるのみで、不明の点がいくつも残されたままであるから本報においてはこれら諸点を究明したところを述べる。

材 料

ウシグソヒトヨ *Coprinus macrorrhizus* Rea f. *microsporus* Hongo (四極性) の下記 6 系統を供試した。

* 岡山大学理学部生物学教室 植物形態学業績 No. 61, 科学研究費 (課題番号 407037) による研究の一部。

** 岡山大学理学部生物学教室 Department of Biology, Faculty of Science, Okayama University, Okayama, Japan.

X, Y 新潟市で松田一郎氏採集
c, d 倉敷市で著者採集
e, f 大津市で本郷次雄氏採集

これらの系統の不和合性因子は、e, f の間に一部共通のものが見られた外は、互いに全く相違していた。これらを2系統ずつ組合わせて実験した場合、一方の不和合性因子を $AaBb$ 、他方のそれを $A'a'B'b'$ であらわすことにする。

実験並びに結果とその考察

両和合性組合わせでは接種した複相菌糸の2核が単相大菌叢の中を核分裂しながら進むとしても、例えば $AB \times (A'B' + a'b')$ の場合、 $A'B'$ 核と $a'b'$ 核とは別々の AB 菌糸の中を移動するか、或いは2核が同一の AB 菌糸の中を移行するのかについては、まだ明らかにされていない。及川の2核移行の結果を考察すると $AB \times (A'B' + a'b')$ で $A'B'$ 、 $a'b'$ の2核は別々の AB 菌糸の中を互いに異なる方向に進み、菌糸の先端に到達したから AB 核と和合し、それぞれ $AB + A'B'$ 、 $AB + a'b'$ の複相菌糸を作ったようにも思われる。Papazian 及び著者は diploidisation を起した大菌叢の周縁に現われた複相菌糸の核移行を調べて、その後の構成を分析したから、及川のような結果を見ることができた。したがって、稀に起こる $A'B' + a'b'$ のような結果から推すと $A'B'$ 、 $a'b'$ の2核は明らかに同一の AB 菌糸の中を移動したように思われる。しかし $A'B' + a'b'$ のような結果は、実は及川の場合のような $AB + A'B'$ 、 $AB + a'b'$ の2種類の複相菌糸がたまたま隣接してでき、この二つが融合して核の交換を行い、その結果 $A'B' + a'b'$ が現われたのではないかと考えることも考えられる。このように複相菌糸同志が融合して、新しい核構成の複相菌糸が生ずるといふことがあつたものかどうかについて次の実験を行った。

殆んど同時期に新らしく得られた c, d, e, f の4系統の複相菌糸を別々の試験管内の培養基に植え、発育中の菌叢の周縁部より菌糸を径3mm大ずつとっては、2系統ずつあらゆる組合わせで並べて植える混合培養を行った。培養基には馬鈴薯薄汁寒天を用い、培養温度は 30°C であった。この混合培養に生じた子実体より単胞子培養を分離し、それらの交配型分析を行って2系統の不和合性因子が混合しているかどうかを検した結果は第1表

第1表 2種類の複相菌糸の混合培養(1)

組合わせ		生じた子実体の
系統	不和合性因子	不和合性因子
c+d=	$AaBb + A'a'B'b'$	$A'a'B'b'$ (=d系統)
c+e=	$AaBb + A'a'B'b'$	$A'a'B'b'$ (=e系統)
c+f=	$AaBb + A'a'B'b'$	$A'a'B'b'$ (=f系統)
d+e=	$AaBb + A'a'B'b'$	$A'a'B'b'$ (=e系統)
d+f=	$AaBb + A'a'B'b'$	$AaBb$ (=d系統)
e+f=	$AaBb + Aa'B'b'$	$AaBb$ (=e系統)

の通りである。

このように、互いの2系統の組合わせ培養においても、生じた子実体の不和合性因子は常にどちらか一方の系統のそれと全く同じであつて、このことは異なる複相菌糸が混在しても、それらの間で核の交換は行われないことを示すものといえる。従つて著者が $AB \times (ab + A'B')$ で得た $ab + A'B'$ の出現複相菌糸は、 ab 、 $A'B'$ の2核が同一の AB 菌糸の中を移行して、その先端に到達したとき、 AB 核をさし置いて移行2核だけで複相菌糸を作ったものであり、 $AB + ab$ 、 $AB + A'B'$ の二つの複相菌糸が核を交換したものではないと考えられる。

上述のように接種した複相菌糸の2核が同一の菌糸の中を此れの場合、及川の推論したように2核の早さに相違があり、常に一方の核が早く進んで先端に達して1菌糸の核と和合し、複相菌糸を作っていくとすれば、diploidisation を起した大菌叢の核移行(核間移行)が同一で、たとへば及川の結果、及び同じ組合わせでは出現複相菌糸の核構成は常に同一であつたという Papazian の結果は一応肯ける。そして移行2核の早さの差が小さく、たまたま菌糸端に2核が同時に到達したという稀な場合にだけ、移行2核で構成された複相菌糸が現われることがあつたと考えられる。

第1表の2系統の複相菌糸組合わせ培養結果をみると、c, d, e, f の4系統の複相菌糸の子実体形成能力に優劣があり、 $e > d > f > c$ のように順位をつけることもできる。しかし、このような結果は単に偶然に得られたものかどうかを確かめるため、更に次の実験を行った。既報(木村 1957b, 第1表)の両和合性組合わせの中から任意に No. 3, 5, 25 を選び、各々において、その組合わせに係のある三つの交配型の菌糸を二つずつ混植培養して3種類の複相菌糸を得た。これらを前記のように

第 2 表 2 種類の複相菌糸の混合培養 (2)

番 号	組 合 わ せ	子実体を形成した菌糸
No. 3 $AB \times (ab + A'B') = AB + A'B'$ について		
(1)	$(AB + ab) + (AB + A'B')$	3 回共に $AB + A'B'$
(2)	$(AB + ab) + (ab + A'B')$	" $AB + ab$
(3)	$(AB + A'B') + (ab + A'B')$	" $AB + A'B'$
No. 5 $a'b' \times (A'B' + ab) = a'b' + ab$ について		
(4)	$(a'b' + A'B') + (a'b' + ab)$	3 回共に $a'b' + ab$
(5)	$(a'b' + A'B') + (A'B' + ab)$	" $a'b' + A'B'$
(6)	$(a'b' + ab) + (A'B' + ab)$	" $A'B' + ab$
No. 25 $ab \times (AB + A'B') = AB + A'B'$ について		
(7)	$(ab + AB) + (ab + A'B')$	3 回共に $ab + AB$
(8)	$(ab + AB) + (AB + A'B')$	2 回は $AB + A'B'$, 1 回は $ab + AB$
(9)	$(ab + A'B') + (AB + A'B')$	3 回共に $AB + A'B'$

等量ずつとって二つ並べて植え、生じた子実体よりの単胞子培養を交配型分析して、どちらの複相菌糸が子実体を作ったかを調べた。同一の組合わせを同時に平行して3回ずつ行ったが、その結果は第2表のようである。

この結果を見ると No. 3 及び No. 25 の各組では、組合わした3種類の複相菌糸の子実体形成能力に順位をつけることができるが、No. 5 の組ではそれができなかった。しかし(1)~(9)の各組合わせ3回の結果は、(8)の組合わせに僅かに例外が見られた外は、常にある一方の菌糸が子実体形成に優先的であることを示した。

このような結果を得ると、一つの疑問が生じて来る。diploidisation で大菌叢の周縁部に現われた複相菌糸の核構成は普通、その複相菌糸の一部をとって培養し、生じた子実体からの単胞子培養の交配型を分析することによって決めるのであるから、例えば $AB \times (ab + A'B')$ で diploidisation を起した AB 菌叢の周縁部からとった複相菌糸の中に、もし $AB + ab$, $AB + A'B'$ の2種類が混在していたとしても、上記の複相菌糸間の子実体形成能力の差から、常に $AB + A'B'$ の方が子実体を作ったのではないかということが疑われる、つまり同一の菌糸の中を ab , $A'B'$ の2核が移行する場合、両者の早さに差があるとしても、どちらか一方が常に早く進むということではなくて、ある菌糸の中では ab 核の方が早く進んで AB 核と和合し、他の菌糸の中では $A'B'$ 核の方が早くて AB 核と和合し、その結果、周縁部には $AB + ab$,

$AB + A'B'$ 、或いは稀れには $ab + A'B'$ まだが混生しているのではなからうか。この点を明らかにするため、次の実験を行った。

今度の実験も第2表に示す No. 3, 5, 25 の組合わせについて行った。既報(木村 1957b, 第1表)の結果は No. 3, 5 では大菌叢の核は他系統の核と和合し、No. 25 では移行2核が和合したことを示した。これらの各組合わせで周縁部に現われた複相菌糸を数ヶ所から少量ずつピンセットでとってペトリ皿の中の無菌水へ一緒に入れ、柄付針で菌糸をよくほぐして菌糸の浮遊液を作った。これをペトリ皿内の寒天培養基上に塗布し、個々の菌糸片の中、発育を始めたものを一つずつ単胞子分離の場合と同じ西門氏法(1938)で分離した。本菌の場合、浮遊液中の菌糸片はそれまでの処理で傷つけられて死ぬものが多いが、生き残ったものも塗布後 30°C で1昼夜位では、まだ活潑な生長を示さない。しかし別の菌糸との混合を防ぐため、なるべく早めに分離した。上記のような菌糸の浮遊液塗布によらず、大菌叢の周縁の複相菌糸端を1本ずつ切りとるということも決して不可能ではないが、もし2種類の複相菌糸が混在しておいて、その中の一方が特に生長が早いという場合は、切りとる菌糸の種類が一方に偏ってしまう恐れがあるから、この方法はさけた。

このようにして前記の3つの組合わせからそれぞれ単菌糸培養5個を得、これらに生じた子実体よりの単胞子培養の交配型分析を行って、各菌糸の核を推定した。その結果は各組合わせよりの 5

個の菌糸は皆同一の核構成を示し、

No. 3 $AB \times (ab + A'B')$ では全部 $AB + A'B'$

No. 5 $a'b' + (A'B' + ab)$ では全部 $a'b' + ab$

No. 25 $ab \times (AB + A'B')$ では全部 $ab + A'B'$

であった。

この結果から見ると周縁部の複相菌糸は1種類
だけであり、従って接種した複相菌糸の2核の進
行速度に差があり、常に一方の核が早く進んで大
菌叢の核と和合したことがうかがえる。No. 3, 5
は既報の結果と今回の単菌糸培養の結果とは一致
しているが、No. 25 では既報の結果のような2
核移行を示したものはあらわれなかった。これは
No. 25 の組み合わせでは $A'B'$ 核の方が早く進むの
ではあるが、 AB 核の早さと大きな差がないと考
えられる。したがって、今回の実験結果からい
うと、多くの $ab + A'B'$ であるが、たまたま $A'B'$ 核
と AB 核とが同時に菌糸端に到達し、これら移行2
核で複相菌糸を作った $AB + A'B'$ 菌糸も僅少な
が、しかし、既報の結果から、これら2核の核
が混在した菌糸も、あり得る。このこと、
第2表(9)の組み合わせで示すように、 $AB + A'B'$
の方が $ab + A'B'$ よりも子実体形成能力が優れて
いるから、これら二つの菌糸の混合培養では AB
+ $A'B'$ の子実体が生ずる筈である。

移行2核が菌糸端に同時に到達するということ
は稀で、従って移行2核をつねに観察し、し
てもその数は少ないものと考えられる。このこと
は No. 25 の単菌糸培養5個の中に移行した2核
をもつ菌糸が全く認められなかったこと、及び
(木村 1957b, 第1, 3表)のように両和合性組
合せ、移行2核が和合した菌糸がほとんどない、
同じ組み合わせでも結果を異ならしめて、結果
が得られ難かったことから推察できる。

上述のように移行する2核に差があること、
間違いないように思われるが、これだけでは説明
できない問題がまだ残されている。前書きで述べ
たように、及川の実験結果では diploidisation を
起した大菌叢は周縁部も内部も同一の核構成であ
った。著者も既報(1957b)の第3表, No. 31~38
の組み合わせでは、diploidisation を起した大菌叢
の周縁部だけでなく、中心部からも菌糸をと
って、その核構成を分析したところ本報の第3表
のように及川の結果と一致した。なお、既報(木
村 1954a)のように diploidisation を起した大菌

叢の内部の菌糸は、鏡検すれば clamp のない単
相菌糸の状態であるが、その中には分裂しながら
移行してきた複相菌糸の核も存在するから、これ
を基として核構成が推定して来るものであるこ
とを附言しておく。

第3表 両和合性組み合わせによる diploidisation

番 号	組 合 わ せ	出現複相菌糸の2核	
		周縁部	中心部
31	$AB \times (ab + A'B')$	$ab + A'B'$	$ab + A'B'$
	"	$AB + A'B'$	$ab + A'B'$
32	$A'B' \times (ab + AB)$	$A'B' + AB$	$A'B' + AB$
33	$AB' \times (ab + A'B)$	$ab + A'B$	$ab + A'B$
34	$A'B \times ab + AB'$	$A'B + AB'$	$A'B + AB'$
35	$ab \times (AB + A'B')$	$AB + A'B'$	$AB + A'B'$
36	$A'B' \times (AB + ab)$	$A'B' + AB$	$A'B' + AB$
37	$aB' \times (AB + A'b)$	$aB' + AB$	$aB' + AB$
38	$A'b \times (AB + aB')$	$A'b + AB$	$A'b + AB$

いま、 $AB \times (A'B' + a'b')$ で周縁部の数ヶ所が
等しく $AB + A'B'$ であっても、移行2核の遅速
で説明がつく。しかし、内部の数ヶ所までも周縁
部と同一の核構成であったことは核の遅速では解
釈ができない。何となれば、 $A'B'$ 核に較べて $a'b'$
核が遅く進むとしても、不和合性組み合わせの場合
の diploidisation の速度から推して、それ程遅い
ものとは考えられず、 $A'B'$ 核が AB の菌糸端に
到達して $AB + A'B'$ となる場合には、 $a'b'$ 核も大菌
叢の中を相当の距離まで進んで来ているものと思
われる。従って、周縁部が同一に $AB + A'B'$ であ
っても、内部からは $AB + A'B'$ の外に $AB +$
 $a'b'$, $A'B' + a'b'$ のような、いろいろな菌糸が出
て来てもよい筈であるのに、及川や著者の結果
は周縁部と内部の核構成が一致するのは何故かと
いう問題が出て来る。

著者は前報(1957b)で核の和合には不和合性
因子の外に、和合の強さを左右する変異因子も関
係することを仮定したが、上記の問題については
次のように解釈をしたい。例えば $AB \times (ab +$
 $A'B')$ の組み合わせで、 AB 核と ab 核は同一の子
実体から由来したものであるから AB 核と ab 核
の間に変異因子の差はない。でも、それは AB 核と
他系統の $A'B'$ 核との差よりも小さい。このよう
な場合、変異因子の差の大きい $A'B'$ 核の方が ab

核よりも早く AB 菌糸の中を移行して菌糸端に達し、全周縁部に $AB+A'B'$ の複相菌糸を作る。一方、大菌叢の内部の菌糸の中には AB , $A'B'$ 核の外に、遅れて進んで来た ab 核も存在するが、この場合 AB 核はやはり変異因子の差が多い $A'B'$ 核と和合して周縁部と同じ $AB+A'B'$ を作る。そして、もし AB , ab , $A'B'$ 3 核の変異因子が仮にそれぞれ $CDEF$, $cdEF$, $CDef$ のようであった場合には AB , ab 間、及び AB , $A'B'$ 間の変異因子の差はどちらも二つずつであるから、 ab 核と $A'B'$ 核の間に早さの差があるとしても、それは僅少であり、従って ab , $A'B'$ 核が同時に AB 菌糸の先端の細胞に到達するという事も稀れにあり、この場合、先端の細胞中には AB , ab , $A'B'$ の 3 核が存在することになる。この中、どの 2 核が和合して複相菌糸を作るかというに、変異因子の差が 4 で、従って和合性が最も強い ab 核と $A'B'$ 核が結びつき、2 核移行を示す菌糸となるのであろう。周縁部がこのような場合、内部においてもまた同じく和合性の最も強い ab と $A'B'$ が対合することは当然である。

以上のように考察すると第 3 表の結果も了解できる。及川の $AB \times (A'B' + a'b')$ のような型の組合わせでは、 $A'B'$, $a'b'$ 2 核は同一子実体から由来したものであっても、変異因子には両者の間に多少の相違があるから、大菌叢の AB 核の変異因子と差の大きい方の核が早く周縁部に達して AB 核と和合し、菌叢の内部では他方を排して同じく AB 核と結びつくものとすれば及川の結果も説明できる。そして $AB+A'B'$, $AB+a'b'$ の 2 種類の複相菌糸が同一菌叢の周縁部に見られた組合わせは $A'B'$, $a'b'$ の 2 核の間に早さに関して殆んど差がなかった場合と考えられる。

このように論じてくると、移行 2 核の早さの相違について疑念が生じるかも知れない。それは $AB \times (ab + A'B')$ で周縁部に $AB+A'B'$ の複相菌糸が出て来た場合、 $A'B'$, ab 2 核のどちらが早いということはなく、2 核は常に同時に AB 菌糸の先端の細胞に到達して大菌叢の内部の菌糸の場合と同じように、 AB , ab , $A'B'$ の 3 核間の和合性の強弱によって常に $AB+A'B'$ となったのではないか、つまり 2 核の進行速度には全く差はなく、単に変異因子の差の大小による和合性の強弱だけによって、すべてが説明できるのではなから

うかということである。著者は前述のように、周縁部の菌糸を 1 本ずつ分離培養して、その核構成を分析した結果から移行速度に遅速のあることを推論したのであって、複相菌糸を作る直前の AB 菌糸の先端の細胞が 2 核であったのか、又は 3 核になっていたのかを観察したわけではない。しかし既報 (木村 1957b) のように、移行 2 核が和合した結果が得られても、同じ組合わせで実験をくりかえしたら、多くの場合、違った結果であったことは上記の疑念に対する一つの反証であろう。著者 (未発表) はまた $AB \times (ab + \underline{A'B'})$ のような和合性組合わせの実験で、 ab , $\underline{A'B'}$ の 2 核はどちらも移行し、しかも両者の間には遅速の差があることを証明する結果を得ているが、両和合性の場合も和合性の場合と同様、2 核の進行に遅速の差があると考えてよいであろう。

不和合性組合わせの場合には、どの菌糸を用いても新核形成という僅少な例外を除いては、不和合性なるが故に必ず 2 核移行を証明する結果を得ることができる (木村, 1957a)。しかし、両和合性組合わせでは接種した複相菌糸の 2 核の中、どちらか一方が少しでも早く菌糸端に到達したら、直ちに大菌叢の核と和合して複相菌糸を作ってしまうから、2 核移行を示す結果はなかなか得られず、単に偶然に出るのを待つより外はない。しかし、 $AB \times (ab + A'B')$ の場合、 AB , ab , $A'B'$ 3 核間の変異因子の差が出来るだけ少くなるようにしてやれば、 ab , $A'B'$ 2 核は同時に菌糸端の細胞に到達し、そして ab , $A'B'$ 間の和合性が AB , ab 間、 AB , $A'B'$ 間のいずれよりも強いという条件が揃う機会が幾分かなりとも多くなりはいしないだろうか。既報 (木村 1957b) の第 2 表は産地を異にする野生子実体から単胞子分離した原菌糸を用いて両和合性組合わせを行った結果であり、第 3 表は第 1 表で組合わした 2 系統の核が融合、減数分裂して生じた次代の菌糸を用いての両和合性の実験結果であるが、2 核移行を示したものは第 2 表では 47 の組合わせの中の 3 (約 6.4%) であり、第 3 表では 38 の中の 6 (約 15.8%) であった。この出現率の差は、実験数が少いから単に偶然のものであるかも知れないが、あるいは前述の予想を幾分裏付けするものとも考えられる。

両和合性組合わせの実験で 2 核移行を示す結果が得られる機会、周縁部に現われた複相菌糸の

2 核を分析するよりも、大菌叢内部の菌糸から生ずる複相菌糸を分析する方がよいと思われる。それは例えば $AB \times (ab + A'B')$ で $A'B'$ 核の方が ab 核よりも早く進んで、周縁部に $AB + A'B'$ の複相菌糸が現われた場合でも、大菌叢内部の菌糸の中には AB 核の外に $A'B'$ 核と遅れて来た ab

核とが存在し、これら3核の中で ab , $A'B'$ 間の和合能が最も強ければ、内部の菌糸をとって培養すると2核移行を示す $ab + A'B'$ 菌糸が生じてくるからである。例えば第3表、組合わせ No. 31 の2回の結果が、このことを示すものといえよう。

Summary

1. The present paper deals with the nuclear migration from the diploid mycelium into the large haploid mycelium in the doubly legitimate combination, using *Coprinus macrorrhizus* f. *microsporus* (a tetrapolar fungus).

2. Both of the nuclei of the diploid mycelium migrate through the same hyphae of the large haplont, in which one of the two nuclei advances more rapidly than the other.

3. The speed of nuclear migration seems to be controlled by the modifiers which also decide the conjugation affinity between two compatible nuclei.

4. The both nuclei of an inoculated diplont can rarely arrive simultaneously at the terminal cell of a haploid hypha, resulting in coexistence of three nuclei in the cell. In this case, if the conjugation affinity between the foreign two nuclei is stronger than that between the nucleus proper to the haplont and either of the foreigners, conjugation will occur between the two foreigners.

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コンブ目植物における孢子囊群の 比較発生学的研究 (予報)*

西林 長朗** • 猪野 俊平**

Takeo NISHIBAYASHI** and Shumpei INOH**: Preliminary
Note on the Sorus Development in Laminariales*

昭和 32 年 10 月 16 日受付

コンブ目植物の孢子囊群は、柵状に並んだ遊走子囊と単細胞の側糸とからできている。これらの孢子囊群の発生学的研究は、Thuret (1850), Setchell (1891), Schrader (1903), Sauvageau (1918), Kylin (1918), McKay (1933), Herbst and Johnstone (1937), Clare and Herbst (1938), Hollenberg (1939) らにより、コンブ目の各種について行われ、Fritsch (1945) がこれらの結果をまとめて、「孢子囊群の発生様式は、すべてのコンブ目植物において本質的に同じである。」といっている。それ故、この事実を確かめる為に、著者らは本邦産のこの目の種について、その孢子囊群発生を観察を計画し、その一部報告は、ツイシコンブ (1956)、スジメ (1957) についてすでに行った。今回は、*Alaria crassifolia* Kjellm. チガイソ、*Laminaria japonica* Aresch. マコンブ、*Undaria pinnatifida* (Harv.) Sur. ワカメの3種について、更に観察を行った。その結果、チガイソと後の2種との間、孢子囊群の発生様式に差異のあることが知られたのでここに予報する。

材 料 と 方 法

本研究に使用した材料は、*Alaria crassifolia* Kjellm. チガイソ、*Laminaria japonica* Aresch. マコンブ、*Undaria pinnatifida* (Harv.) Sur. ワカメの3種であり、チガイソは1954年8月中旬、マコンブは同年7月下旬に北海道大学理学部附属

室蘭海藻研究所附近で採集したものであり、ワカメは1956年6月上旬、瀬戸内海の香川県向笠島で採集したものである。採集後、成熟適期の孢子葉を切り出し、これを細かく切って、阿部氏液で20~40時間固定した。その後、普通のパラフィン切片法により、4~5 μ の切片を作り、10%過酸化水素水で約40時間漂白した後、ハイデンハイン氏鉄明礬ヘマトキシリンで染色し観察を行った。

観 察

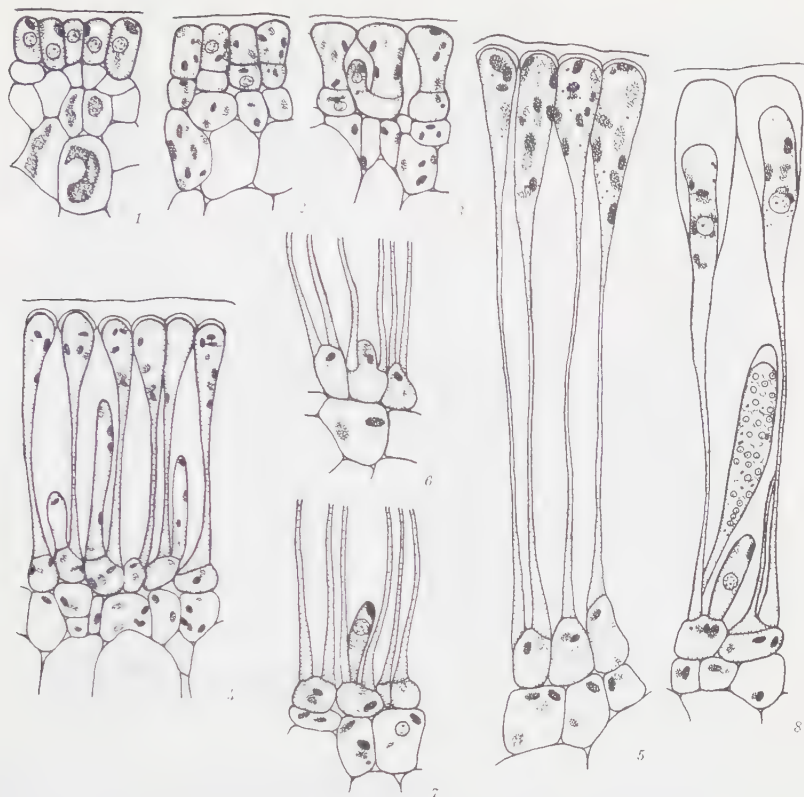
1. *Alaria crassifolia* Kjellm. チガイソ

チガイソの孢子囊群は、コンブなどのように普通の葉の面にはできないで、下部の茎の両側に羽状に並んでいる特別な小葉、すなわち孢子葉の両面に作られる。この場合、孢子囊群が働き始めると、その部分は黄褐色に変色する。

未成熟の孢子葉は、1層に規則正しく並んだ表層 (meristoderm) と、皮層、髓の3部よりなっている。表層の細胞は1核と数箇の色素体を含んでいるが、成熟期に達するとその細胞の一つずつが、葉面に平行な膜により、外側の上位細胞 (upper cell) と内側の下位細胞 (lower cell) とに各々分けられる (Figs. 1, 2)。その後、上位細胞は伸長して単細胞側糸となるが、この細胞がいくらか伸長した時に、下位細胞はこの若い側糸の間に突起を出す (Fig. 3)。間もなく下位細胞の核は分裂するが、その中の1核は色素体とともに突起の中へ移る。その後、突起は下位細胞から隔膜により区別される。この区切られた突起は、その後、核分裂を行うことなく伸長し、すでにできている側糸の間に入り込み、この両者は全く区別すること

* 岡山大学理学部生物学教室植物形態学研究業績 No. 64。玉野臨海実験所業績 No. 44。

** 岡山大学理学部生物学教室 Department of Biology, Faculty of Science, Okayama University, Okayama, Japan.



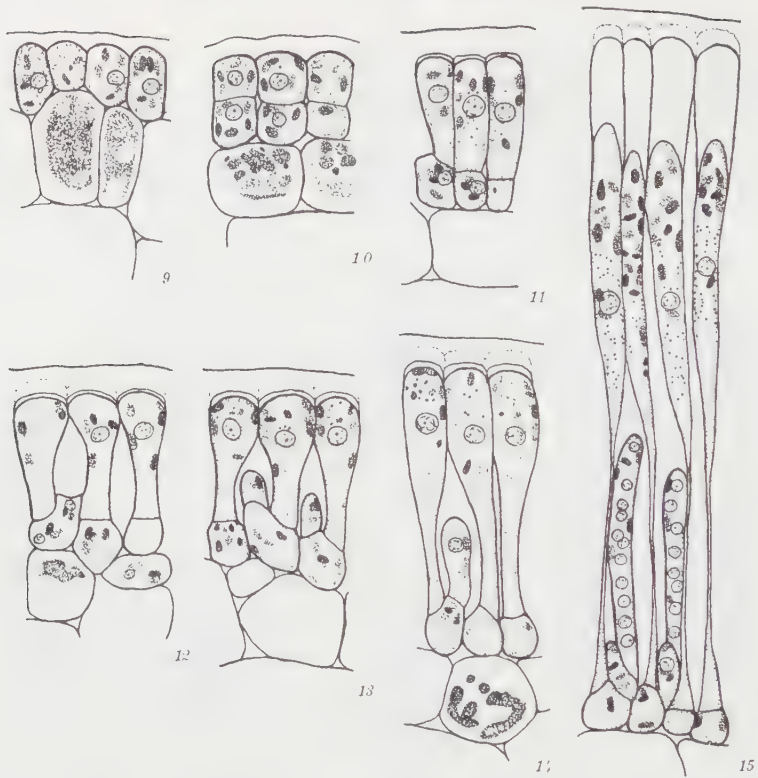
Figs. 1-8. Development of sorus of *Alaria crassifolia* Kjellm. All magnifications ca. $\times 650$.

Fig. 1. Section of sporophyll in sterile portion, showing one layered meristoderm. Fig. 2. Transverse division of the meristoderm into the upper cell and lower cell. Fig. 3. Projection of the lower cell between the young paraphyses. Fig. 4. Growth of the paraphyses. Fig. 5. Completed paraphyses. Fig. 6. Projection of the lower cell between the completed paraphyses. Fig. 7. Growth of the zoospore-mother-cell. Fig. 8. Further stage of development.

ができなくなる (Figs. 4, 5)。それ故、一つの下位細胞の上に、上位細胞が生じてきた筈と、下位細胞の突起から生長した側糸との2つの細胞が並ぶようになる。その後、これらの側糸は生長を続け、その頭部が膨らみ、外膜も肥厚してくるが、基部は細くなって糸状となる。このようにして側糸ができあがった後に、下位細胞は再びこれらの側糸の間に突起を出し、突起はある程度大きくなった時、下位細胞から区切られて、これが遊走子母細胞となる (Figs. 6, 7)。一つの下位細胞から二つの遊走子母細胞が形成されている場合も観察された。完成した側糸の頭部は三角形状を呈し、その中には1核と数箇の色素体が含まれている。側糸の外膜はいくらか肥厚しているが、さほど顕著ではない (Fig. 8)。

2. *Laminaria japonica* Aresch. マコンプ

マコンプの孢子嚢群は、最初、葉の裏面に円形の環紋となつて形成されるが、後には表面にも作られる。葉は表層、皮層、髓の3部よりなっているが、マコンプの皮層細胞は比較的大きく、多くの場合、その中に同化産物を含んでいる。1層に整然と並んでいる表層細胞は1核と数箇の色素体を含んでいるが、成熟期になると表層細胞は横裂して、外側の上位細胞と内側の下位細胞とに分けられる (Figs. 9, 10)。上位細胞はそのまま伸長して単細胞側糸となる。上位細胞が幾分、伸長して細長くなった時、下位細胞はこの若い側糸の間に突起を出し始める (Figs. 11, 12)。この頃から、側糸の外膜は肥厚してくる。下位細胞の核は分裂して2核となるが、この中の1核は下位細胞の中



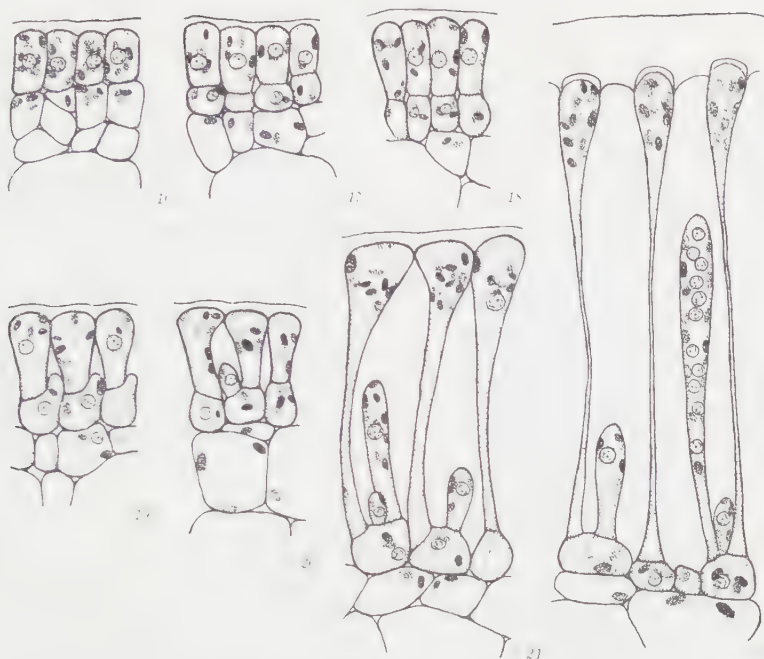
Figs. 9-15. Development of sorus of *Laminaria japonica* Aresch. All magnifications ca. $\times 650$.

Fig. 9. One layered meristoderm. Fig. 10. Transverse division of the meristoderm to form the lower cell and the future paraphysis. Fig. 11. Growth of the paraphyses. Fig. 12. Projection of the lower cell between the adjacent young paraphyses. Fig. 13. Transverse division of the lower cell to form the zoospore-mother-cell. Figs. 14, 15. Further stages of development.

央に留まり、他の1核は1箇または2箇の色素体とともに突起の方へ移動する。核および色素体が突起の中へ完全に入り込んだ時、突起は隔膜により下位細胞からしきられて、これが遊走子母細胞となる (Fig 13)。すなわち、下位細胞は側糸を形成することなく、直ちに遊走子母細胞を切り出している。その後、遊走子母細胞も側糸とともに生長して行くが、生長にともなって側糸の外膜は著しく肥厚する。側糸の頭部は細長い棍棒状を呈しているが、基部は傘状となり、その間に遊走子嚢は生長する (Fig. 14)。一つの下位細胞から二つの遊走子嚢が切り出されているのが、屢々観察される。完成した側糸の頭部には、中央に一つの核が、核の周辺には色素体および、同化産物と思われる多数の小さい顆粒が存在する (Fig. 15)。

3. *Undaria pinnatifida* (Harv.) Sur. ワカメ

ワカメはチガイソと同じく、下部の莖の両側に特別な胞子葉をつける。胞子葉は褶状をなし、その表裏両面に胞子嚢群は作られる。胞子葉の表層細胞は1層に規則正しく並び、その各細胞内には1核と数箇の色素体が含まれている (Fig. 16)。成熟期になると、表層細胞は葉面に平行な膜により、外側の上位細胞と内側の下位細胞とに分けられる (Fig. 17)。上位細胞はその後、伸長を続け単細胞側糸となる。上位細胞がいくらか伸長したとき、下位細胞はこれらの若い側糸の間に突起を出し、突起がある程度大きくなったとき、突起は隔膜により下位細胞から切り出され、これが遊走子母細胞となる (Figs. 18, 19, 20)。その後、側糸の頭部は生長にともなって大きくなるが、基部



Figs. 16-22. Development of sorus of *Undaria pinnatifida* (Harv.) Sur.
All magnifications ca. $\times 650$.

Fig. 16. One layered meristoderm. Fig. 17. Transverse division of the meristoderm to form the lower cell and future paraphysis. Fig. 18. Growth of the paraphyses. Fig. 19. Projection of the lower cell between the young paraphyses. Fig. 20. Transverse division of the lower cell to form the zoospore-mother-cell. Figs. 21, 22 Further stages of development.

は徐々に細くなり、遂には糸状となる (Fig. 21)。遊走子母細胞がある程度大きくなると、下位細胞は再び別の遊走子母細胞を切り出し、かくして一つの下位細胞から二つの遊走子母細胞が形成される。完成した側糸の端に三角突起を呈し、その中には1核と数箇の脂肪体が含まれている。側糸の外膜の肥厚は僅かであるが、最上層の細胞角皮は非常に厚くなっている (Fig. 22)。

考 察

以上の観察結果から、チガイソ、マコンブ、ワカメの3種の遊走子嚢および側糸はともに、その表層 (meristoderm) の細胞から発生することが明らかになった。しかし、その発生様式の細部は、マコンブとワカメとは似ているが、チガイソは他の2種と違っていた。マコンブおよびワカメでは、表層細胞の上下二分によって生じた上位細胞はそのまま側糸になるが、下位細胞は直ちに突

起を生じ、この突起が区切られて遊走子母細胞となる。すなわち、側糸と遊走子母細胞とは同時に発生する。チガイソでは、表層細胞の上下二分によって生じた2細胞の一つである上位細胞は、前種らと同じくそのまま側糸になるが、他の一つ下位細胞からの突起はワカメおよびマコンブのように、遊走子嚢にならないで、そのまま伸びてまた側糸になってしまう。そして、この両側糸が伸長しきった時期に、改めて、先きに側糸を出した下位細胞に突起が起り、これが遊走子母細胞となる。それ故、チガイソでは側糸は一次に、遊走子母細胞は二次に発生し、両者は同時に発生しない。以上述べたように、ワカメ、マコンブらとチガイソとの発生様式の相違は、生殖細胞の分化する時期にあるわけで、ワカメ、マコンブでは表層細胞の第一回の上下二分の時にその分化をみ、上位細胞から生殖に関係なき側糸、下位細胞から遊走子母細胞が発生する。それに対してチガイソでは、表層細胞の第一回の分裂によって生じた上下

の細胞とともに側糸を作るので、その時期には未だ生殖細胞は決定されない。そうして、次の下位細胞の第二回目の分裂の時に、初めて遊走子母細胞となつて生殖細胞を作る。それ故、生殖細胞の分化は、ワカメおよびマコンブではチガイソよりも早い時期に決定される。

結局、チガイソはワカメやマコンブよりも、生殖に関係ない側糸細胞を一回だけ余計に作り出すことになり、生殖機構からいえば、一つだけ異なる、いかいえ、生殖に関係のない細胞を作ることになる。この点で、チガイソはワカメやマコンブよりも一つおくれた種のように思われる。なお、小さいことであるが、側糸細胞の形に差異があって、マコンブではその外膜の肥厚が著しく、

頭部は棍棒状を呈しているが、ワカメおよびチガイソでは外膜の肥厚はマコンブほど著しくなく頭部は三角形状を呈している。マコンブの孢子囊群の発生様式および成熟した側糸の形態は、先に著者らが報告したミツイシコンブ(1956)に非常によく似ており、その間には差異を見出すことができなかった。

本研究を行うに当り、材料の採集、実験に多大の便宜をいただいた、北海道大学理学部附属室蘭海藻研究所所長山田幸男博士、ならびに中村義輝博士、岡山大学理学部附属玉野臨海実験所所長川口四郎博士に厚く御礼申し上げます。

Summary

The development of sorus of *Alaria crassifolia* Kjellm., *Laminaria japonica* Aresch. and *Undaria pinnatifida* (Harv.) Sur. has been studied. In these species both the zoosporangia and paraphyses originate from the meristoderm of the sporophyll. But, there are some differences in the mode of development of sorus between *A. crassifolia* and other two species. In *L. japonica* and *U. pinnatifida*, the upper cell is destined to be paraphysis and the lower cell to produce zoospore-mother-cells, and zoosporangia and paraphyses develop simultaneously. In *A. crassifolia* the upper cell is destined to be paraphysis but the lower cell to produce both the paraphysis and zoospore-mother-cells, and the development of zoosporangia takes place secondarily after the complete growth of paraphyses.

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日本植物学会会則

(昭和 31 年 7 月 14 日改正)

第1条 本会は、1944年12月25日、

第2条 本会は植物学の進歩と普及をはかり、あ
らわけて会員おたが、いのしたしなを増進の
とする。

第3条 本会は前条の目的を達するために「植物学雑誌」その他の出版物の刊行、大会・講演会・講習会などの開催、その他必要と認めらる事業を行う。

第4条 本会の会員は次の5種とする：

通常會員・終身會員・特別會員・外國通信會員・名譽會員

いい、終身会員とは所定の終身会費を納めたものをいう。

第6条 特別会員とは引き続き本会の会員であつて、
本会に対していちじるしい功芳のあつた者、外
国通信会員とは本会に関係の深い外国人、また
名誉会員とは植物学に対して功芳のいちじるし
い者で、いづれも評議員会で協議し会長が総会
で推薦し承認された者をいう。但しやむを得な
い場合は、あつて総会の承認を求めることがあ
る。これらの会員は会費を要しない。

第7条 本会に、臨時評議員を置き、臨時評議員は、評議員の
かの選挙文書に属するものから、選挙文書に

第8条 本会には次の役員を置く：

会長 1名・幹事長 1名・幹事 若干名・
評議員 若干名・編集委員 若干名

第9条 役員は会員の中から選出し、任期は2カ
年とする。但し重任することができる。選出に
ついての規定は別に設ける。

・10 幹事（理事会）の仕事をする。幹事長は、会長を助けて会務を処理する。幹事は庶務・会計・編集・図書管理など日常の会務を行う。

第 11 条 評議員は評議員会を構成する。評議員
会は会長の諮問の範囲で本会の要務を審議し、
また、その執行を監督する。

第 12 条 編集委員は編集委員会を構成する。幹事、理事及び常務理事は編集委員会が「植物学雑誌」の編集に関しての一切の責任を負う。

第 13 条 本会の会計年度は 1 月に始まり 12 月

第14条 本会は原則として毎年1回総会を開き、以下の事項を議決する。なお会長が必要と認めた場合には、臨時総会を開くことができる。

第15巻「日本と世界の青年会を同じ研究発表などを行う。大会には大会会長そのほか若干名の
(裏面へつづく)

入会申込書

入会の申込、会費（年 900円）の払込、そのほか会へのご連絡のあて先は：

東京都文京区東京大学農学部植物学教室内 日本植物学会会館。

それから会費の払込は振替貯金口座東京 11190 番を利用されるのが最も便利です。なお振替でお払込の場合は特に領収書をさし上げませんからあしからず。

臨時の役員を置くことができる。正会会長は会長が推薦し、そのほかの役員は正会会長が依嘱する。

第 16 条 会員は会合に出席して講演をし議事に参加し、「植物学雑誌」に投稿し、また本会所有の図書を読覧することができる。また毎号無料にて「植物学雑誌」の配布を受ける。

第 17 条 会員が退会しようとするときは、そのことを本会に通知しなければならない。もし会

費の滞納があるときはその際全額を納めなければならない。但し既に納めた会費は一切これを返さない。通常会員が会費を滞納したときは直ちに前条の権利を停止し、1 年以上滞納したときは除名することがある。

第 18 条 本会の会則または付則を変更するには総会または臨時総会でこれを協議し、出席会員の 3 分の 2 以上の同意を得なければならない。

付則第 1 会 費（会則第 5 条関係）

第 1 条 通常会員の会費は年 900 円とし 300 円ずつ分納することもできる。終身会費は 15,000 円とする。

このほか国外在住会員に限り植物学雑誌の送

料を負担する。

第 2 条 評議員編集委員以外の役員は在任中会費を要しない。

付則第 2 地方支部（会則第 7 条関係）

第 1 条 地方支部は原則として 50 名以上の会員のある地方に置き、北海道・東北・関東・北陸・中部・近畿・中国四国・九州の 8 とする。

第 2 条 会員は居住地の支部に入るのが原則であるが、事情により他の支部に属することもでき

る。

第 3 条 支部には支部長を置く。支部長は支部を代表する。

第 4 条 そのほかの規定は各支部ごとに設ける。

付則第 3 役員を選出方法（会則第 9 条関係）

第 1 条 会長は全会員の投票で選出される。その際評議員会は若干名の候補者を推薦することができる。

第 2 条 評議員は各支部別に支部会員によって選出される。その定員は各支部最低 2 名とし、会員数が 100 名を越える支部では 50 名までごと

に 1 名を増す。評議員は引続き 3 期選出されることはできない。なお会長および幹事長は評議員を兼任することができない。

第 3 条 幹事長・幹事・編集委員はいずれも会長が依嘱する。

..... き り と り 線

入会や転居などの場合には必ず別に支部へも連絡して下さい。支部のあて先は次のとおりです。なおどの支部へ入るかは大体地理的にきまるわけですが、ご都合で A 支部よりも B 支部の方が便利だという方は B 支部に入られてもよいことになっています。

北海道支部	札幌市北 8 条西 5 丁目 北海道大学理学部植物学教室
東北支部	仙台市片平町 東北大学理学部生物学教室
関東支部	東京都文京区白山御殿町 東京大学附属植物園
北陸支部	金沢市仙石町 金沢大学理学部植物学教室
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Cytological and Morphological Studies on the Gametophytes of Ferns XI. The Influences of Neutral Salts and Hydrogen Ion on the Duration of Life of the Fern-Spermatozoid

by Isami IGURA*

伊倉伊三美：ニダ類の配子体に関する細胞学的並びに形態学的研究 XI.
シダ類精子の生存時間に及ぼす中性塩及び水素イオンの影響

Received December 10, 1957

The chemotaxis of spermatozoids was studied by Shibata (1902, 1905, 1911) in *Salvinia* and some ferns, and recently by Rothschild (1952) in *Pteridium*. Fujii and Asahina (1952) investigated the discharges of the *Isoetes*-spermatozoids caused through the influences of ethyl ether and other chemicals. According to Du Buy and Nuernbergk (1938), several authors (Buller 1900, Lidforss 1905, Bruchmann 1909) had studied the chemotaxis of fern-spermatozoid caused by the different chemicals, especially the salts of organic acids.

Concerning the influences of neutral salts or hydrogen ion concentrations, Yamaha (1946) carried out the experiments on the vital staining of various cells of several plants and Igura (1954) on the vital staining of fern-prothallia. However, their influences on the duration of life of the spermatozoids are presumed to be significant and important in the physiology of fertilization in plants. From this viewpoint the present work was undertaken.

Materials and Methods

The spermatozoids of the following ferns were used as materials: *Athyrium melanolepis* Christ, *Equisetum arvense* L., *Osmunda japonica* Thunberg, *Plagiogyria Matsumureana* Makino, *Athyrium pycnosorum* Christ, *Matteuccia struthiopteris* Todaro, and *Osmunda cinnamomea* L. var. *asiatica* Fernald. The former four species were mainly applied to the test of the neutral salts and the latter three to those of the hydrogen ions. For the purpose of confirming the effects of cations on the duration of life of the spermatozoid, the aqueous solutions of chlorides such as KCl, NaCl, BaCl₂, CaCl₂, MgCl₂, SrCl₂, AlCl₃, FeCl₃ were used as the media, whose concentrations were made in the various grades from 5×10^{-3} to 0.08 M chiefly at the intervals of 0.02 M. The buffer-solutions which were held in the hard glass bottles,

* Biological Institute, Faculty of Education, Yamagata University, Yamagata, Japan. 山形大学教育学部生物学教室

were prepared according to McIlvaine's or Kolthoff's table and their pH-values were from 2.2 to 9.0 at the intervals of 0.2. The concentrations of the buffer-solution were made to be various grades (the ions contained in these buffer-solutions were considered to be neglected). The redistilled water was used in all the cases. The microscopic observation was made under the illumination of the light source whose candle power was about 1200 LX, and the temperatures of room and water in the experiment were kept to be 20° and 18° respectively.

The paraffin was set at the both sides of a drop of medium on the slide glass, in which the prothallia were dipped and the cover glass was put on the drop and the paraffin and the border of cover glass was sealed with paraffin. According to this method, the concentration of the medium on the slide glass was able to be kept constantly for a long time during the observation. Thus the preparation was observed under the phase contrast microscope. In the solutions used as the media, the duration of movement of the spermatozoid which was extruded out of the spermatid was measured by a stop watch and the duration of movement was regarded as the life-time of spermatozoid (Yuasa 1933, 1938, Igura 1950)¹⁾. Namely, three to five minutes after the spermatozooids swam out of the spermatids, the prothallia were removed and the mean value of the longest time was obtained, during which the spermatozooids began to swim and all spermatozooids stopped their movements.

Results

It is not easy, in fact, to ascertain the stop of movement by tracing only one spermatozoid and it is doubtful whether the stop of movement means the death of spermatozoid or not. That is, even after the stop of movement, the spermatozoid may continue to live. As stated before, however, it was regarded that the duration of movement means the duration of life of the spermatozoid in the present study. On the other hand, in the study of the duration of life of the spermatozoid, the various conditions of the medium, in which the spermatozoid lives, e. g. acidity, osmotic pressure, concentration of ions, contents of various gases, temperature, light and others, must be considered (Yuasa 1937). Besides these habitat factors, the individual variation of the duration of life is also important, even in the same habitat-factors.

The results of observations concerning the duration of life of the spermatozoid are given in the following Table 1.

In all the media, the more the concentration of the solution increases, the more the duration of life decreases. Thus, the limit concentrations of solutions used as the media, in which the spermatozooids are able to live, are summarised as Table 2.

1) As stated later, it is difficult to determine precisely the duration of life, but usually the duration of movement means the duration of life.

Table 1. The duration (minutes) of life of the spermatozoid in the media of the neutral salts. (Room temperature, 20°; water temperature, 18°)

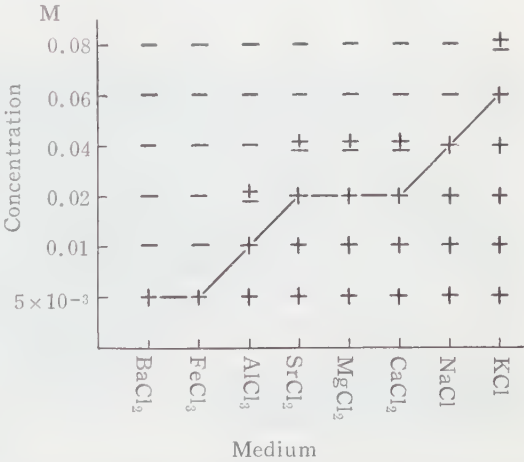
M	Salt							
	KCl [*]	NaCl [△]	BaCl [△] ₂	CaCl [○] ₂	MgCl [△] ₂	SrCl [△] ₂	AlCl [△] ₃	FeCl [△] ₃
5 × 10 ⁻³	31-33	30-35	6	53-58	40-43	35-40	50-55	4-5
0.01	30-35	22-27	×	25-30	28-32	29-32	37-42	×
0.02	18-20	11-16	×	9	6	8	8-13	×
0.04	15-20	5-10	×	2	3	4	×	×
0.06	10-15	×	×	×	×	×	×	×
0.08	×	×	×	×	×	×	×	×
Exp.	I [*]	II [△]	III [△]	IV [○]	V [△]	VI [△]	VII [△]	VIII [△]
Redistilled H ₂ O	92-97	66-70	68-73	122-127	108-110	95-100	74-78	65-70

^{*}, *Equisetum arvense* L.; ○, *Plagiogyria Matsumureana* Makino; △, *Athyrium melanolepis* Christ; ×, the spermatozoids are immobile; Exp., experiment.

Considering the influences of the neutral salts on the duration of life of the spermatozoid shown in Table 2, it is found that the limit concentration of solution is highest in the case of KCl and lowest in that of FeCl₃, and the more the ion-value of the solution becomes high, the less the limit of concentration; i. e. the series of ion (cation) is as follows, K⁺, Na⁺>Ca⁺⁺, Mg⁺⁺, Sr⁺⁺, >Al⁺⁺⁺>Fe⁺⁺⁺.

The manners of the movements of spermatozoids in the solutions whose concentrations are lower than the limit one are same in the cases of the observations by Yuasa (1933) and Igura (1950) which were made in the tap water. When the concentration of solution becomes higher than the limit one, the spermatids tend to discharge out of the antheridium as small masses. Though they scatter gradually, the spermatozoids do not swim out of the spermatids. Even if the spermatozoid swims out of the spermatid in the solution whose concentration is high, its number is small, its movement is slow in general, and the duration of movement is short. Even when the movement is

Table 2. The limit concentrations of the solutions of neutral salts within which the spermatozoids are able to live (*Athyrium melanolepis* Christ). (Room temperature, 20°; water temperature 18°)



-, the spermatozoid is immobile; ±, the small number of spermatozoid move for a short time; +, the spermatozoids move.

active, it suddenly loses its velocity and stops, and sometimes it makes a rotatory movement in a right-handed direction or often in a left-handed.

As to the influences of the hydrogen ion on the duration of life of the spermatozoid, the results shown in the following Table 3 are obtained.

Table 3. The influence of the hydrogen ion concentration on the duration of life of the spermatozoid. (Room temperature, 20°; water temperature, 18°).

The figures (except those of pH-values) in the table represent the duration-times (minutes) within which the spermatozoids are able to live.

pH-value	M	McIlvaine-buffer: <i>Matteuccia struthiopteris</i> Todaro						Kolthoff-buffer: <i>Osmunda cinnamomea</i> L. var. <i>asiatica</i> Fernald					
		0.1	0.05	0.025	0.01	5×10^{-3}	1×10^{-3}	0.1	0.05	0.025	0.01	5×10^{-3}	1×10^{-3}
9.0		×△	×△	▲10	15	20	30	×△	×△	×	10	15	20
8.2		×	×	15	20	40	50	×	×	10	20	35	50
7.2		×	×	15	30	30	45	×	▲10	15	20	35	40
6.0		×	×	▲10	20	20	35	×	×	▲15	20	20	40
5.2		×△	×	▲10	15	15	30	×△	×	▲10	10	20	25
4.2		×△	×△	▲10	15	15	20	×△	×△	×△	▲10	15	20
3.0		×△	×△	×△	▲10	▲10	▲10	×△	×△	×△	▲10	▲10	▲15
2.2		×△	×△	×△	×△	×△	▲10	×△	×△	×△	▲10	▲10	▲10

×, the antheridium discharges but the spermatozoid does not swim out of the spermatid; △, the number of the discharged antheridia is small; ▲, the number of spermatozoids which move is small.

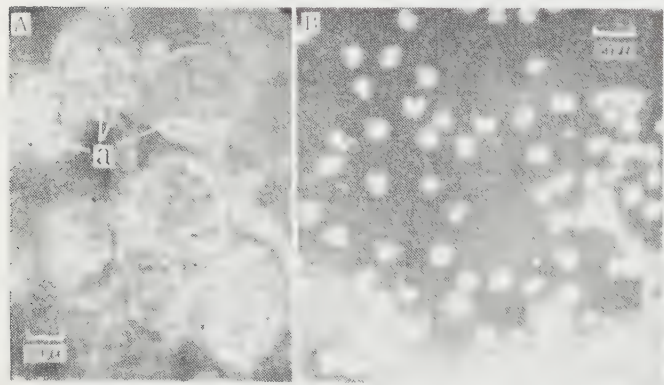


Fig. 1. The antheridia and spermatozoids of *Matteuccia struthiopteris* Todaro under the negative high objective of the phase contrast microscope.

A. Antheridia in which the spermatozoids (a) are included (Upper view). B. Spermatozoids in the solution of 1×10^{-3} M McIlvaine-buffer whose pH-value is 8.2; 45 minutes elapsed after they extruded out of the antheridia; some of them swam and some stopped their movements.

The duration of life of the spermatozoid is longest in both 1×10^{-3} M McIlvaine's and Kolthoff's buffer-solutions whose pH-values are 8.2 in both cases, and the mean value is 49 minutes (Fig. 1). The test in the tap water which was carried out at the same time showed that the duration of life is 39 minutes which is shorter than that of the former. The duration of life is fairly longer in 1×10^{-3} M buffer-solu-

tion whose pH-value is 5.2-9.0, but longer at pH 6.0-8.2. It is found that the near value of pH 8.2, that is, the alkaline side is optimum for the life of spermatozoid.

The smaller the pH-value becomes, the shorter the duration of life becomes gradually, and the number of spermatozooids which are able to move is small. In other words, the hydrogen ion is presumed to have an injurious effect on the life of spermatozoid. The limit concentration of the buffer-solution related to the movement of spermatozoid was found to be in the near value of 0.025 M. The smaller the concentration becomes than this value, the more suitable it becomes for the spermatozoid to live. The state of the movement of spermatozoid resembles the case of the neutral salts.

The duration of life of the spermatozoid is influenced, as above-mentioned, by the habitat-factors, but even in the same factors, it shows the individual variation. The results obtained

in regard to the variation in the duration of life of the spermatozoid (*Athyrium pycnosorum* Christ) in the solution of 1×10^{-3} M Kolthoff-buffer (pH 8.2) which showed the longest duration, are given in Fig. 2.

Fig. 2. The individual variation of the duration of life in 234 spermatozooids of *Athyrium pycnosorum*, Christ (Room temperature, 20°; water temperature, 18°; 1×10^{-3} M Kolthoff-buffer, pH 8.2).

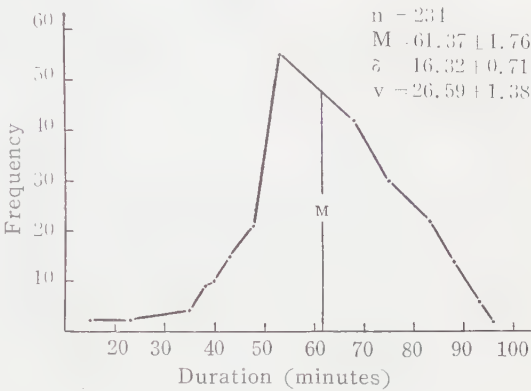


Table 2. The limit concentrations of the solutions of neutral salts within which the spermatozooids are able to live (*Athyrium melanolepis* Christ).

(Room temperature, 20°; water temperature, 18°)

Duration (minutes)	15	23	35	38	40	43	48	53	68	75	83	88	93	96	Total
Frequency	2	2	4	9	10	15	21	55	42	30	22	14	6	2	234

Considerations

The life of spermatozoid is influenced by the ion-value (cation) in the neutral salts in inverse proportion to the largeness of the ion-value, and the hydrogen ion disturbs the life of spermatozoid, therefore, the spermatozoid is able to continue its life for a longer time in the medium whose pH-value is large, namely whose acidity is in the alkaline side. The studies concerning the chemotaxis of spermatozoid by Shibata (1902, 1905, 1911), Rothschild (1952) and other authors were carried out in consideration of the hydrogen ion in the medium, by which the taxis occurs. In writer's opinion, however, when the spermatozooids extrude out of the spermatids and swim about in the medium on the prothallia, it is favorable that the ion-value of cation in the medium is small and its acidity is alkaline. Moreover, when the spermatozoid arrives at the archegonium through the medium and approaches to the egg-cell, the same conditions must be kept. In this way, it is

significant and important from a standpoint of the physiology of fertilization to know the influences of the neutral salts and the hydrogen ion on the duration of life of the spermatozoid.

Summary

Studying the influences of the aqueous solution of KCl and other seven kinds of the neutral salts and the hydrogen ion on the duration of life of the spermatozoid of *Athyrium melanolepis* Christ and other six species of Pteridophyta, the following facts were known.

1. The duration of life of the spermatozoid is effected by the cation and the more the ion-value of cation increases, the more the limit concentration of the solution in which the spermatozoid lives decrease gradually.

2. The hydrogen ion disturbs the duration of life of the spermatozoid. The medium whose acidity is alkaline (pH 8.2) is most favorable for the duration of life of the spermatozoid.

3. In fertilization, when the spermatozoids approach to the archegonium and enter in it and at last reach the egg-cell, it is favorable that the ion-value of cation in the medium is small and the acidity of medium is alkaline, in which the spermatozoids move on. This fact is significant and important from the viewpoint of the physiology of fertilization.

The writer expresses his hearty gratitudes to Prof. A. Yuasa, University of Tokyo, who gave him valuable instructions in the course of this work and kindness in revising the manuscript.

Literature

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The Degradation of Rutin by an Imperfect Fungus of the Genus *Pullularia*

by Shizuo HATTORI* and Ichio NOGUCHI*

服部静夫*・野口市夫*: 不完全菌 *Pullularia* による rutin の分解

Received December 14, 1957

Introduction

There have been comparatively few studies on the metabolism of rutin in the animal body (1-4) and among them the work of C.W. Murray and his coworkers (2), that 3, 4-dihydroxyphenyl acetic acid was found in the urine of rabbits after oral administration of rutin or quercetin, is very interesting as to *in vivo* degradation of rutin or quercetin. As regards microorganisms, however, there has been hitherto no study whether and how rutin would be metabolized by them.

In our laboratory, we found one day in June this year, that, among uncovered solutions of gallic acid, phloroglucinol, and rutin, only the latter one had been infected after a few days by a fungus, which was revealed by inoculating it into a cultural medium. Curiously enough the presence of the fungus could not be anticipated when seen by naked eyes, but after 3 days culture an apparent growth of a fungus was eventually observed. The isolation was, however, relatively easy and we obtained a pure culture with the cultural solution given in the experimental part.

In the present paper, some of the results obtained in the study on the degradation of rutin by this fungus is presented. So as to make the description easily understandable, we would like to express this fungus as *Pullularia***.

The *Pullularia* forms phloroglucinol and protocatechuic acid from quercetin when cultivated in rutin solution to about 75 % of the theoretical value, while rutinose, rhamnose, and glucose were formed as the products of hydrolytic process of rutin by means of rhamnodiastase and rhamnosidase.

Experimental

The organism was at first grown at 25° on a medium containing 0.5 % glucose, 0.5 % pepton and 2 % agar, the pH of the medium being adjusted to 6.0 with phos-

* Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo. 東京大学理学部植物学教室

** The genus to which this fungus belongs has not yet been definitely determined, but, according to the studies of Mr. M. Yoneyama, Biological Laboratory, Minami Branch, Hiroshima University, it has been so far decided to belong to *Pullularia*. The morphological and taxonomical research are now being made by him, and we are looking forward to seeing his successful result in near future.

phate buffer, and the concentration of phosphate in the medium being 0.2 %. After 48 hrs growing, a small amount of the mycelium (about 2.5 mg. in wet weight) was added to 50 ml of a 0.02 % aqueous solution of rutin, which had been previously sterilized on a boiling water-bath for 1 hr, and allowed to stand at 25° for 5 days.

For isolation and identification of the degradation products, extracts of the rutin solution, which had been made by shaking several times with ether, were subjected to ascending paper chromatography (Whatman No. 1 filter paper), using *n*-butanol-acetic acid-water (4:1:2) or 80% phenol as solvents. The detection of the location of the various hydroxy aromatic substances on the air-dried paper was accomplished by spraying a solution of freshly prepared benzidin reagent or a saturated aqueous ferric chloride solution.

On the other hand, for quantitative analysis of the metabolic products, ultra-violet absorption of the cultural medium of rutin was measured with a Beckman spectrophotometer and paper chromatography was used, too.

Rutin used in this work was extracted from the dried unopened flowers of *Sophora japonica* and purified by repeated recrystallisation.

Benzidin reagent was prepared as follows. Benzidin (5 g) was stirred with conc. HCl (14 ml) and water (980 ml) was added afterwards to the suspension. A suitable amount of this solution was mixed with an equal volume of a 10 % NaNO₂ solution and the mixture was stirred till it became clear and pale yellow (5).

For isolation and determination of saccharides, a sample was subjected to ascending chromatography on paper (Whatman No. 1), using upper phase of *n*-butanol-acetic acid-water (4:1:5) as solvent. The detection of the location of saccharides was accomplished by spraying benzidin reagent containing trichloroacetic acid.

Saccharides were prepared by concentrating the cultural medium under reduced pressure, from which aromatic compounds previously had been removed by shaking with ether.

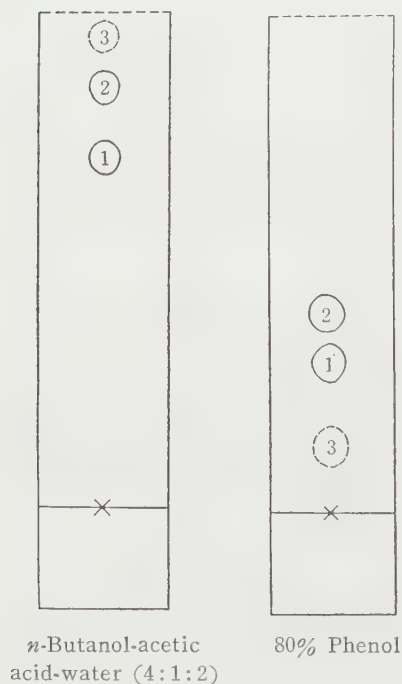


Fig. 1. Paper chromatograms of ether extract of rutin solution cultured with the *Pullularia*.

- Spot 1.....phloroglucinol
- Spot 2.....protocatechuic acid
- Spot 3.....unknown substance

Results

A schematic diagram of a chromatogram of an ether extract of the medium, in which 0.2 mg. of rutin per ml was cultured with the *Pullularia* for 5 days at 25°, is shown in Fig. 1.

Spot 1 was red in color after spraying benzidin reagent, but colorless with an aqueous ferric chloride solution, and its R_f value agreed exactly with that of phloroglucinol both in butanol and in phenol solvents.

Spot 2 yielded yellow orange dyes with benzidin reagent and blue dyes specific to protocatechuic acid after spraying an aqueous ferric chloride solution. Its R_f value coincided with that of protocatechuic acid both in butanol and in phenol solvents.

It is obvious from these results that spots 1 and 2 demonstrate the presence of phloroglucinol and protocatechuic acid, respectively, in metabolic products of rutin. In addition to these spots, a third spot, small in size, was detected. It showed a red color with benzidin reagent, but its identification has not yet been successful.

An ultraviolet absorption spectrum of the rutin solution after degradation with the *Pullularia* is shown in Fig. 2. If rutin is perfectly decomposed to phloroglucinol and protocatechuic acid, the sample used in drawing curve 1 has to contain 0.0011 mg. of phloroglucinol and 0.013 mg. of protocatechuic acid both per 1 ml.

It is thought to be effected by the other metabolic products that the absorption shown in curve 1 (Fig. 2) is higher than that of theoretical in wave lengths from 240 m μ to 265 m μ .

About 75 % of the theoretical quantity of protocatechuic acid seems to be present in the medium judging from the absorption at 290 m μ wave lengths, where it seemed not almost to be effected by other metabolites. On the other hand, from comparison of the size and color of the spot on paper

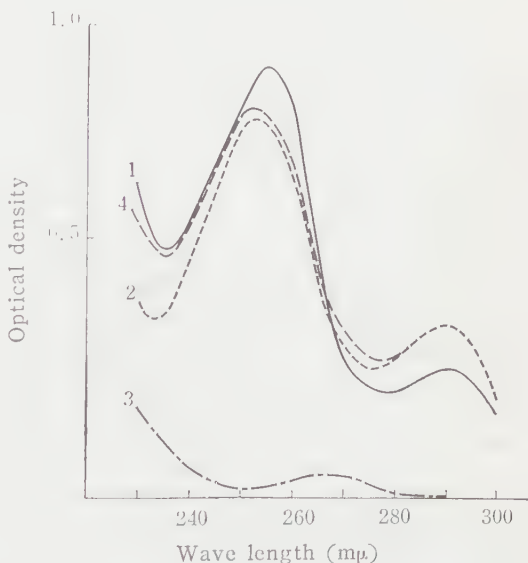


Fig. 2. The ultraviolet absorption spectra of rutin solution cultured with the *Pullularia*.

- Curve 1. Rutin solution (containing 0.18 mg. rutin/ml) cultured with the *Pullularia*; 3.5 times diluted with water.
- Curve 2. Protocatechuic acid; aqueous solution (0.013 mg./ml)
- Curve 3. Phloroglucinol; aqueous solution (0.011 mg/ml)
- Curve 4. Protocatechuic acid (0.013 mg./ml) and phloroglucinol (0.011 mg./ml); aqueous solution.

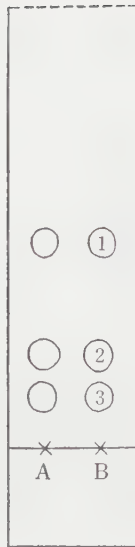
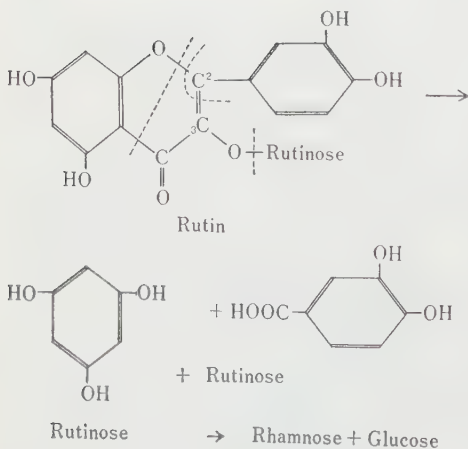


Fig. 3. Paper chromatogram of saccharides in rutin solution cultured with the *Pullularia*.

Spot 1.....rhamnose
 Spot 2.....glucose
 Spot 3.....rutinose
 Asample
 Bcontrol
 Solvent ...upper phase of *n*-butanol-acetic water (4:1:5)



chromatogram with those of pure compounds, more than about 70 % of the theoretical quantities of both protocatechuic acid and phloroglucinol were established to be present in the cultural medium.

A schematic diagram of a chromatogram of the saccharides in the cultural medium containing rutin is shown in Fig. 3.

It is evident from Fig. 3 that rutinose, rhamnose and glucose are present in the cultural medium. From this result, the activities of rhamnosidase and rhamnodiastase are very probable to be present in the microorganism.

Discussion

The finding of the fungus *Pullularia* which is able to decompose rutin to phloroglucinol and protocatechuic acid is of interest. From the results, rutin may be degraded as follows. This scheme reminds us of the alkaline degradation of quercetin that proceeds quite in the same way. In this connection the way of rutin degradation taking place in the cultural solution of the fungus seems to be similar to pure chemical process. The fate of C₃ and C₄ of the aglycone quercetin is also unknown as is the case with chemical hydrolysis.

In the case of degradation of rutin in animal body, 3, 4-dihydroxyphenyl acetic acid has been found, but there is detected no such substance in the degradation products of rutin by this *Pullularia*.

Though rutin is rather easily decomposed by the *Pullularia*, quercetin itself does not show any sign of decomposition, when alone subjected to the action of the organism. The degradation of quercetin, however, occurs, when sucrose was added to the cultural solution. The studies on the kinetics of the interesting and curious disruption of quercetin by the *Pullularia* are now in progress.

Acknowledgment

We greatly appreciate the enthusiastic co-operation of Mr. M. Yoneyama in making manifold experiments for determination of the imperfect fungus.

Summary

1. A microorganism, an imperfect fungus, was isolated from the air, which decomposes quercetin, the aglycone of rutin, into phloroglucinol and protocatechuic acid, when cultured with rutin, but not with quercetin itself. This fungus belongs to *Pullularia*.

2. More than about 70 % quantity of theoretical value of phloroglucinol and protocatechuic acid are found after decomposition of rutin by the organism.

3. The activities of rhamnodiastase and rhamnosidase are found in the organism and rutinose, rhamnose and glucose are detected in the medium after 5 days culture.

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Studies on Cross Compatibility of Diploid, Triploid and Tetraploid Barleys I. Seed Development in Reciprocal Crosses between Diploids and Induced Autotetraploids

by Takumi TSUCHIYA*

土屋 工*: オオムギにおける二倍体三倍体, および四倍体間の変雑親和性. 第1報.
二倍体と同質四倍体間の正逆交雑における種子稔性と次代の染色体数

Received December 16, 1957

The present author has succeeded in producing in barley a considerable number of autotriploids during the past several years (Tsuchiya 1949, 1952a, b, 1953a). In the present paper, the experimental results regarding cross compatibility between diploid and induced autotetraploid barleys are described.

Materials and Methods

In the crossing experiments, diploids and induced autotetraploids of the following 5 cultivated varieties and 1 wild species were used. All autotetraploids used were produced by Dr. T. Ono by colchicine treatments (Ono 1946, 1947).

Hordeum sativum Jessen (cultivated varieties)

Early Golden Melon (two-rowed)

Hakata No. 2 (")

Hosokara No. 2 (six-rowed)

Hosomugi (")

Mochimugi (")

H. spontaneum C. Koch var. *transcaspicum* Vav. (two-rowed wild species)

Bonnett's method (Bonnett 1938) was used exclusively in the crossing experiments. To count the chromosome numbers in root tip cells, both paraffin method and aceto-carmines squashes were employed and the meiotic chromosomes were observed in temporary aceto-carmines preparations.

Results and Considerations

1) Crossing experiments

Adopting Bonnett's method (l. c.), the flowers were opened by slitting the lemma with a sharpened forceps. Through the slit made in the lemma the anthers were removed and pollinations were made. The results of the crosses are given in Table 1.

* Kihara Institute for Biological Research, Yokohama, Japan. 木原生物学研究所

Table 1. Cross fertility in reciprocal crosses of $4x \times 2x$ and germination of the seeds.

Materials and cross combin.	Year	Number of		Fertility (%)	Germination	
		florets	seeds ¹⁾		No. of seed	%
$4x \times 2x$ EGM. ²⁾	1947	94	3	3.19	1	33.33
	1952	49	6	12.24	0	—
	1953	370	274	74.05	1	0.36
HST. ³⁾	1952	793	130	16.39	12 ⁴⁾	9.23
	1953	956	468	48.95	12 ⁵⁾	2.56
	1952	77	1	1.29	0	—
Hakata No. 2	1953	206	63	30.58	2	3.17
	1952	47	0	0.00	—	—
Hosomugi	1952	80	0	0.00	—	—
Hosokara No. 2	1953	49	20	40.81	1 ⁶⁾	5.00
Mochimugi	1948	19	18	94.73	2 ⁷⁾	11.11
$2x \times 4x$ HST.	1952	21	19	90.48	0	—
Hakata No. 2	1952	40	39	97.50	0	—
Hosomugi	1952	16	0	0.00	—	—

1) Plump and shrivelled (parthenocarpic) seeds are involved.

2) Early Golden Melon.

3) *Hordeum spontaneum* C. Koch var. *transcaspicum* Vav.

4) 7 plants died in early seedling stage and the remaining 5 grew to maturity.

5) 1 plant died in the seedling stage and the remaining 11 grew to maturity.

6) Died early in the seedling stage.

7) 1 plant died early in the seedling stage and the remaining 1 matured.

No seeds were obtained from the crosses $4x \times 2x$ or $2x \times 4x$ in Hosomugi and $4x \times 2x$ in Hosokara No. 2, while many seeds were obtained from $4x \times 2x$ in Early Golden Melon. The cross of Early Golden Melon $4x \times H. spontaneum$ var. *transcaspicum* $2x$ (1948) gave a very high percentage of seed setting (94.7 %). From the cross $2x \times 4x$ (1952) in *H. spontaneum* var. *transcaspicum*, 19 seeds were obtained from 21 florets (90.5 %). In the reciprocal cross of 1952, $4x \times 2x$, the fertility was lower than in 1953. Hakata No. 2 showed a rather low percentage of seed setting in the cross $4x \times 2x$. The fertility, however, showed an increase from 1.3 % in 1952 to 30.6 % in 1953. The reciprocal cross, $2x \times 4x$, showed the highest fertility (97.5 %) in 1952. Mochimugi showed a fertility of 40.8 % in the cross $4x \times 2x$ in 1953.

2) Germination tests

The results of germination tests are given in the last 2 columns of Table 1. Though the seed setting was rather good in the cross $2x \times 4x$, combinations of *H. spontaneum* var. *transcaspicum* and Hakata No. 2, the seeds did not germinate.

Out of 283 seeds of the cross $4x \times 2x$ in Early Golden Melon, two triploid plants were obtained, namely 1 plant out of 3 seeds in 1947 and 1 out of 274 seeds in 1953, but all the 6 seeds obtained in 1952 failed to germinate.

Considerable number of seeds obtained in the cross $4x \times 2x$ of *H. spontaneum* var. *transcaspicum* germinated. In 1952, 12 out of 130 seeds germinated; 7 plants died in an early seedling stage and the remaining 5 grew to maturity (Tsuchiya 1953a). 12 seedlings emerged from 468 seeds in 1953 but 1 died by an accident and the remaining 11 plants grew up to maturity.

In Hakata No. 2, 1 seed of the cross $4x \times 2x$ (1952) did not germinate. Two seeds out of 63 obtained from the same cross in 1953 germinated and the plants matured.

Mochimugi gave one seedling from 20 seeds in the cross $4x \times 2x$ in 1953, which was killed by an accident in an early stage.

The results mentioned above show that seeds obtained in the crossing with diploid mother plants, $2x \times 4x$, do not germinate, though seed setting is very good, with the exception of Hosomugi. The situation is reversed in the reciprocal cross, $4x \times 2x$; that is, seeds were able to germinate in many cases, although seed fertility and percentage of germination were not always high. Similar relationship has been reported in *Raphanus* (Nishiyama 1949, 1952), *Brassica* (Nishiyama and Inamori 1952, 1953), *Capsicum* (Nishiyama and Karasawa 1954), *Hordeum* and *Zea* (Håkansson 1953), and other plants.

The fact that seed setting in selfing of diploids and induced autotetraploids of barley is very good shows that both pollen and embryo-sacs of diploids and tetraploids are functional (cf. Ono 1946, 1947, 1948, 1949a, b, 1952, Tsuchiya 1953b). However, only a few seeds obtained from the cross $4x \times 2x$ germinated and none of the seeds from the reciprocal cross, $2x \times 4x$. These results seem to show that the cross incompatibility between diploid and autotetraploid barleys may be ascribed mainly to zygotic sterility and not to gametic abortion.

Håkansson (1953) ascribed the failure of seed setting in reciprocal crosses between diploid and tetraploid plants of barley (and maize) to the "degeneration of endosperm and embryo" resulted from the unbalanced quantitative relations of chromosome numbers (genomes) within the seeds. According to him, "the changed relations between the maternal tissue and the endosperm were considered to be the initial cause of the irregular development and degeneration of the endosperm; the death of the embryo was secondary to the disturbed endosperm development". The significant differences seen in either seed setting or in germination percentage among materials and in different years, however, remain unsolved problem.

Smith (1951) and Håkansson (l. c.) reported that the production of autotriploids in barley by crossing induced autotetraploids with diploids was very difficult. The present experiments carried out during the past 4 years tend to confirm their results, namely only 19 autotriploids were obtained from the 2721 ($4x \times 2x$) and 77 ($2x \times 4x$) florets, i. e., 0.67 %.

3) Cytological observations

Among 31 plants obtained from the seeds of the cross $4x \times 2x$, 10 died in early seedling stage and the chromosome numbers of remaining 21 plants were counted as given in Table 2.

Table 2. Chromosome number of plants obtained from the cross $4x \times 2x$ in barley

Materials	Year	Number of plants with respective chromosome numbers of			
		20	21 29	30
Early Golden Melon	1947 ¹⁾	1			
"	1953		1		
<i>H. spont.</i> var. <i>transcaspicum</i>	1952 ²⁾	1	3	1	
"	1953		10		1
Hakata No. 2	1953		2		
EGM. ³⁾ $4x \times$ HST. ⁴⁾ $2x$	1948 ⁵⁾		1		

1) Tsuchiya 1949, 1952a.

2) ——— 1953a.

3) Early Golden Melon.

4) *Hordeum spontaneum* C. Koch var. *transcaspicum* Vav.

5) Tsuchiya 1952b.

One plant of Early Golden Melon obtained from the cross $4x \times 2x$ in 1947 was a hypo-triploid with $2n=20$ chromosomes (Tsuchiya 1949, 1952a) and another plant from the same cross in 1953 had $2n=21$ chromosomes.

Among 5 matured plants of $4x \times 2x$ (1952) in *H. spontaneum* var. *transcaspicum* there were 3 eu-triploid with $2n=21$, 1 hypo-triploid with $2n=20$, and 1 hyper-tetraploid with $2n=29$ chromosomes. Somatic chromosomes of all 11 surviving plants from the cross $4x \times 2x$ in the same species (1953) were counted; 1 was hyper-tetraploid with $2n=30$ and the remaining 10 were eu-triploids with $2n=21$ chromosomes.

Two individuals from the cross $4x \times 2x$ of Hakata No. 2 made in 1953 were eu-triploid with $2n=21$ chromosomes. The hybrid plant raised from the cross Early Golden Melon $4x \times$ *H. spontaneum* var. *transcaspicum* $2x$ in 1948 was also eu-triploid with $2n=21$ chromosomes (Tsuchiya 1952b).

As described above, almost of all the plants obtained from the cross $4x \times 2x$ were hypo- or eu-triploids with $2n=20$ or 21 chromosomes. Unexpectedly, however, 29- and 30-chromosome plants were obtained from the cross $4x \times 2x$ in *H. spontaneum* var. *transcaspicum*. These two plants might have resulted from the union of hyperdiploid eggs in tetraploids and unreduced pollen which occur rarely in diploid barley as observed by Smith (1942) and Tsuchiya (1955).

Self-pollination resulting from unsuccessful castration should be taken into consideration as an alternative.

Similar cases of unexpected occurrence of tetraploid plants in the crosses between autotetraploids and diploids have been reported in *Raphanus* (Nishiyama

1949, 1952) and *Brassica* (Nishiyama 1953).

Eight seedlings (27.6 %) out of 31 obtained from the cross $4x \times 2x$ died in a very early stage. From the morphological features these seedlings were assumed to be triploid or nearly triploid. The difficulty in obtaining autotriploids from the cross $4x \times 2x$ in barley may partly be ascribed to a very low viability in early seedling stages.

Summary

1. Diploids and induced autotetraploids of 2 two-rowed and 3 six-rowed cultivated varieties of *Hordeum sativum* and a two-rowed wild species, *H. spontaneum* C. Koch var. *transcaspicum* Vav. were used in the crossing experiments.

2. Seventeen eu-triploids with $2n=21$ and 2 hypo-triploids with $2n=20$ chromosomes were obtained from a total of 2721 florets of the crosses $4x \times 2x$.

3. The cross $2x \times 4x$ gave a high percentage of seed setting with an exception of Hosomugi in 1952, the seeds, however, did not germinate.

4. Unexpectedly, two hyper-tetraploids, $2n=29$ and 30 , were obtained from the cross $4x \times 2x$ in *H. spontaneum* C. Koch var. *transcaspicum* Vav.

5. The percentage of seed setting and of germination differed in different cross combinations and different years.

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Developmental Mechanics of Fucaceous Algae IX. Fates of the Abnormally Cloven *Coccophora* Eggs

by Singo NAKAZAWA*

中沢信午*: フークス科藻類の発生力学 IX. 異常卵割したスギモクの卵の運命

Received January 6, 1958

According to Tahara (9), *Sargassum enerve* and *S. Horneri* eggs are cloven irregularly in various degrees if they are treated with hypertonic sea water soon after fertilization, nevertheless their developmental fate is not altered but they develop to normal embryos. The same phenomenon was discovered by Nakazawa (6) in strongly centrifuged eggs of *Sargassum tortile*. It is also certain that in *Sargassum confusum* eggs some irregular cleavage patterns were derived under natural conditions but their irregularity could not modify their original polarity axis (5). On the other hand, it is reported that when *Funaria* (2) and fern (3) spores are cultured in colchicine, chloral hydrate, or in α -naphthalene acetic acid, irregular germination does occur so that the spores become to be mere masses of cells and they never perform specific morphogenesis so far as they are reared in the same medium. Here arises a question as to why in *Sargassum* eggs the irregular cleavage cannot affect the normal differentiation, while in *Funaria* or in some fern spores it results in the loss of differentiation potentialities. Herein, the writer presents his recent experiments on some abnormally cloven eggs of *Coccophora* to clarify the above question.

In April, 1956 and 1957, *Coccophora Langsdorfii* was collected at Asamushi, eggs discharged in glass vessels were fertilized artificially, and cultured in normal filtered sea water contained in Petri dishes. During this cultivation, abnormally cloven eggs were obtained from among the normal eggs in a proportion of about one per cent. These were obtained not by treatment with a special agent but were derived under natural conditions. Their abnormality could be classified into 1) abnormally segmented eggs, i.e. abnormal merely in their irregular cell wall formation but of normal morphology (Fig. 1), and 2) irregular-formed eggs, i.e. irregular in their external morphology

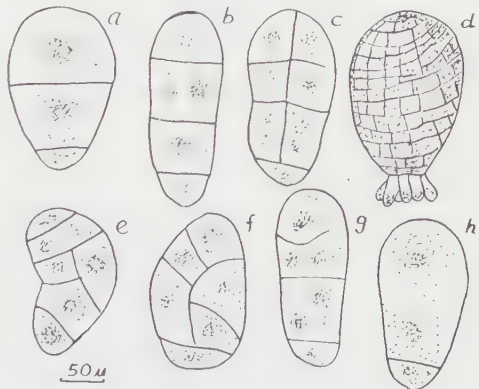


Fig. 1. a) Normal egg, b, c, e-h) abnormally segmented eggs, and d) normal embryo of *Coccophora*.

* Biology Department, Yamagata University, Yamagata, Japan. 山形大学生物学教室.



Fig. 2. Irregular-formed *Coccophora* eggs.

from the latter culture with neutral red for revealing the permeability gradient. For this purpose, 0.1 per cent distilled water solutions of each dye were prepared, then the solutions were diluted into the filtered sea water, pH 8.2, in a proportion of 3 drops to 10 cc of sea water. The staining media thus prepared were separately kept in Petri dishes of 3 cm in diameter and were made 5 mm in depth. In these media the eggs were soaked and inspected with microscope. The remaining abnormal eggs were cultured further on and their later development was recorded. The same experiment was carried out on the normal eggs for comparison. The experiments were conducted in 1956 and in 1957 and the same results were obtained.

Polar staining appeared in 48 to 56 per cent of the abnormally segmented eggs (Table 1). The ratio was much lower than that which was derived in the normal eggs, but it is much higher than what was observed in the irregular-formed

Table 1. Polar vital staining in the normal and the abnormally cloven *Coccophora* eggs. (Experiments in 1957)

Dye	Abnormally segmented eggs		Irregular-formed eggs		Normal eggs	
	Stained per cent	Eggs experimented	Stained per cent	Eggs experimented	Stained per cent	Eggs experimented
Brilliant green	56.4	39	14.2	21	85	100
Neutral red	48.7	45	9.5	20	83	100

eggs. In the normal egg, the polar staining pattern appeared in connection with the egg form as was revealed previously (7). That is, the staining begins to appear at the pointed end where the rhizoid primordia are to be formed later. In the abnormal eggs, however, it does not always appear in relation to the morphology, nevertheless, it is the same as in the normal eggs that the staining is liable to begin at the part where the permeability is highest. Because, the polar

(Fig. 2). 124 of the former and 62 of the latter abnormal eggs were picked up and cultured separately in Petri dishes with filtered sea water at room temperature, about 15°. 39 from the former and 21 from the latter culture were stained vitally with brilliant green, and 45 from the former and 20

staining appeared at a part gradually extends over all the egg protoplasm and at last the egg is uniformly stained. That is, the polar staining cannot be attributed to the differential distribution of a stainable substance but it must be explained from differential entrance of the dye. Young embryos developed in a high percentage from the normal eggs, while the ratio was much lower in the abnormal eggs (Table 2). But a remarkable difference in the embryo formation ratio between the two types of the abnormal eggs should not be neglected. That is, only

Table 2. Normal embryo formation in the normal and the abnormally cloven *Coccophora* eggs. (Experiments in 1957)

Abnormally segmented eggs		Irregular-formed eggs		Normal eggs	
Embryo formation per cent	Eggs experimented	Embryo formation per cent	Eggs experimented	Embryo formation per cent	Eggs experimented
76.9	40	4.7	21	98	100

one, i.e. 4.7 per cent, out of 21 irregular-formed eggs developed to a young embryo, while 30, i.e. 76.9 per cent, young embryos were derived from out of 40 abnormally segmented eggs. The polarity axis, represented by the direction of the formation of rhizoids, was determined in coincidence with the primary external morphology of the egg, that is, the longitudinal axis of the egg became the longitudinal axis of the embryo.

From observations above, it is clear that the developmental polarity cannot be affected by any abnormal segmentation occurred in the egg, but it is controlled by the morphologically longitudinal axis of the egg, and if the external morphology is altered irregularly by some means the morphological potency can be diminished. The failure in differentiation found in abnormally cloven spores of *Funaria* (2) and *Dryopteris* (3) cultured in colchicine, chloral hydrate, or α -naphthalene acetic acid can be explained from the same point of view. However, as to the recovery of their ability of differentiation when returned to normal culture medium should be considered otherwise. Thus it seems that any abnormal segmentation, so far as it is an internal affair, cannot result in loss of the wholeness but the egg still preserves morphogenetic potentialities. This strongly supports an opinion that the main factor of the polarity determination must not be in the internal structure but it must be in the cortical part as has been advanced by Bünning (1), Motomura (4), Nakazawa (8) and by others. When vitally stained, a dye permeability gradient which was reported by Nakazawa (7) was also observed along the longitudinal axis in normal eggs, while this coincidence was not always observed in the abnormal eggs. Therefore it seems that the permeability gradient and the polarity axis are sometimes independent of each other,

while they are closely connected in the normal egg.

Summary

Abnormally cloven eggs of *Coccophora Langsdorfii* were obtained after being fertilized artificially. They were divided into two groups, i.e. abnormal in segmentation and irregular in form. Of these, the following was revealed.

(1) The abnormally segmented eggs can develop to normal embryos, while its ratio is a little lower than that in the normal eggs. The irregular-formed eggs, however, can merely proceed their cell division forming irregular patterns but cannot start morphogenesis.

(2) Polar vital staining, which occurred in the normal eggs, was also observed in the abnormally segmented eggs with brilliant green or with neutrel red. However, different from the normal eggs, the connection between the morphological axis and the staining gradient could not be discerned. In the irregular-formed eggs, the polar staining was not or was hardly observable.

(3) The abnormally segmented eggs can develop into normal embryos, whereas the irregular-formed eggs can merely proceed their cell division but cannot differentiate themselves morphogenetically.

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数種の沈水植物細胞における 硝酸銀還元反応の検討*

吉 田 吉 男**

Yoshio YOSHIDA**: Some Informations on the Silver Nitrate
Reduction in the Cells of Several Submerged Plants*

昭和32年12月6日受付

種々の生体組織に著しい硝酸銀還元能のある事は古くから注目せられ、Szent-Györgyi (1928¹⁾)以来種々の研究結果からその還元要因が Ascorbic acid であることが決定せられ、更に Giroud et Leblond (1937²⁾) 等はこの反応から逆にアスコルビン酸の組織化学的検出法を確立した。

植物細胞では特に生きている葉緑体において硝酸銀を還元して黒くなる反応の強い事が知られ所謂“Molisch”反応として多くの研究者により追求されて来た。しかしながらより慎重な検討がなされている点も少くはない。

ここでは *Elodea* その他の沈水植物の葉の細胞におけるこの反応を検討し、ペーパークロマトグラフィーによりその還元産物の分析を行った結果、若干の知見を得たので報告する。

材 料 と 方 法

Elodea densa Casp., *E. canadensis* Michx., *Hydrilla verticillata* Casp., *Ceratophyllum demersum* L., *Potamogeton crispus* L. の5種の沈水植物の新生葉を被検材料として次の試薬及び処理について夫々の反応程度を調査した。① 弱酸性、アンモニア性、中性の10%硝酸銀液を試薬とし次の諸種の処理を行った。① Giroud-Leblond 法：酢酸々性10%硝酸銀液(pH≈3)に30分浸漬、D.W. で洗滌、チオ硫酸ソーダ3.5%溶液に1時間浸漬、D.W. で洗滌、Glycerin で検鏡、i. これらの手順を暗黒中にて行う。ii. 普通室内散光中にて行う。② 酢酸々性、アンモニア性、中性各硝酸銀液(pH≈3~9 各段階)単独処理(30分~1時間浸漬)、i. 暗黒中にて行う。ii. 普通散光中にて行う。

て行う。③ 試薬をスライド上の被検材料に滴下しカバーをかけ直ちに火焰を通して加熱す。

ペーパークロマトグラフィー：一次元上昇法、東洋ろ紙 No. 50, 2×40 cm を用い、展開剤は n-ブタノール 4, 氷酢酸 1, 水 5, を分液ろと中にてよく混和し2~3日間放置後上澄液を使用する。被検液の調製は新鮮草体 1.5g に5%メタ磷酸1ccを加え乳鉢中で磨砕、遠心分離し上澄液を用いる。ろ紙の下端から7cmを出発線とし、その中央点に0.02 ccの検液を滴下し、乾燥後スタンドに装置し下端1cm迄浸るように展開液を注入、酵素作用を抑制するため器底に少量のKCNの結晶を投入し出発線より30cmの高さ迄展開上昇せしめ、乾燥後試薬を吹きつけ発色せしめる。顯色剤としては上記各硝酸銀液の他 2,6-dichlorophenolindophenol 400 mg/l 50% ethanol 溶液(アスコルビン酸の検出)、Benzidin 0.5 g, 酢酸 5 cc, 純アルコール 20 cc の混液(100~105° に5~15分間加熱処理、還元糖の検出)、その他を使用した。

実 験 結 果

Giroud-Leblond 法：暗処理では液胞、細胞質が一様に淡く暗灰色を呈し、葉緑体は僅かに暗褐色を帯びる。明処理でも殆ど同じ反応を示し、暗と明とで特に差は認められない。

弱酸性試薬(pH≈3)：液胞、細胞質は一様に暗灰色を呈し、葉緑体は弱く黒ずんできた。

アンモニア性試薬(pH≈9)：綿毛状暗灰色沈殿が液胞を充たす。しかし葉緑体の着色は殆んど負に近い。

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** 新潟大学理学部生物予教室 Department of Biology, Faculty of Science, Niigata University, Niigata, Japan.

中性試薬 (pH=6): 液胞は幾分暗灰色を帯び又綿毛状沈殿も若干生ずる。葉緑体は多少黒化す。

Table 1. The reaction intensities in various silver-nitrate reagents.

	Ground-Leblond's procedure			Acidic reagent			Neutral reagent			Ammoniacal reagent		
	in dark	in light		in dark	in light	heating	in dark	in light	heating	in dark	in light	heating
Chloroplast	+	+	weak dark brown	+	+	+++ specific blackening	+	+	+++ specific blackening	-	-	-
Vacuole and cytoplasm	+++	+++	diffused dark grey coloration and flocculum precipitation	+++	+++	diffused weaker darkening	+++	+++	diffused weaker darkening	over all complete darkening with remarkable flocculum precipitation	over all complete darkening with remarkable flocculum precipitation	over all complete darkening with remarkable flocculum precipitation

これらの反応は暗処理と明処理とで殆んど大差は認められない。

加熱処理: 何れの試薬でも加熱によって著しく反応が強められるが、酸性及び中性試薬で加熱処理を行った場合において特に葉緑体が殆んど特異的に強く黒化し、液胞の一樣暗化も弱く、綿毛状沈殿も微弱である (Table 1)。酸性と中性とではその当量が後者に於て優れ、又調製に簡便であるから以後の実験はすべて中性試薬加熱処理法によった。

又従来報告せられた高等陸生植物では典型的反応の場合、grana にまず、還元銀による最も強い黒化が見られることが多かったが、此等沈水植物細胞ではすべて、葉緑体の黒化は殆んど一樣であって、grana に特に著しい知見を得ることはできなかった。

細胞及び葉緑体の vital activity と還元能:

でんぷん量 — 中性試薬加熱処理によって生ずる葉緑体の黒化の強さは一葉でも部分によって強弱あり、それはヨード試験による葉緑体内同化でんぷん量に略々平行であった。即ち中肋、葉脈或は所謂 Blattfält 等のでんぷん量の著しい葉緑体は極めて強く典型的な黒化を示し、液胞中の綿毛状沈殿は少く、同化でんぷん量の少い縁辺、基部等の細胞の葉緑体ではでんぷん量の減少に従って漸次顕著な黒色から次第に灰褐色へと反応がうすくなっている。

傷害 — 機械的であると化学的であるとを問わず傷害をうけ、死滅した細胞では葉緑体は勿論細胞質、液胞にも全く何らの反応をも生じない。生死両細胞を対比する時その contrast は極めて顕著である。

原形質分離 — 0.6M ショ糖液で処理した細胞は強く原形質分離を起すがそれ自体は反応の生起には全く無影響であった。しかし原形質分離処理が長期間に亘る時は細胞が不健全状態に傾くのは当然であって、硝酸銀反応も漸時減衰した。

核の存否による葉緑体の変化と硝酸銀反応 — Elodea では原形質分離処理により一細胞内において原形体が二塊に分割されることがあり、そのような場合核を含む分割塊中の葉緑体は次第に退化して黄化、矮小化し同化でんぷん量も激減するが、核を含まない他方の分割塊中の葉緑体は鮮緑を保って却って肥大化し著しく同化でんぷんを

Table 2. The coloration and intensity of silver-nitrate reaction in the nucleated and enucleated protoplasmic fragments cultured with the 0.6 M sugar solution. The leaf cell of *Elodea densa*.

Cultur duration (days)		2	3	4	5	6	7
Nucleated part	Chloroplast	++++ black	++++ black with slight brown	+++ blackish	++ brown	+	+ - yellowish brown
	Morphological alteration		shrink,	etiolate,	and starch decrease.		
	Vacuole and cytoplasm	dark grey	flocculum	precipitation		faintly	
Enucleated part	Chloroplast	++++ black	++++ black	++++ black	+++ black	++ black	++ black
	Morphological alteration		enlarge,	green,	and starch increase.		
	Vacuole and cytoplasm	dark grey	flocculum	precipitation		faintly	

著りすることは既に報告したが、このような葉緑体の変化と硝酸銀反応との関係を検した。その結果は第2表の如くであって有核部では葉緑体の変化、葉緑素量、同化でんぷん量に全く一時的葉緑体の反発は、から、初期に褐色、葉緑体と著しく減衰し、液胞内の暗灰色綿毛状沈殿も4~5日以後は殆んど見られなくなった。これに対比して無核部では、同化でんぷんは速く保たれたが葉緑体の肥大変化とは平行して増大するわけなく、やはり健全時に比べれば若干減衰の傾向を示した。しかしその程度は前者における程は、前者のcontrastは顕著であった。

暗培養と反応 — *Elodea*, *Hydrilla* を植えた水鉢を暗黒中に保った。既に成育していた葉は暗黒中におかれても葉緑体は30日後でも健全時に比しそれ程顕著な黄化は起さない。しかし同化でんぷんは消費し尽され硝酸銀反応の強さも著しく低下し僅かに茶褐色を呈するに止まる。しかし全く負となることはなく生死両細胞を対比する時生きている限り反応能が保たれる事は明かである。又暗保中に新に発した葉は無論著しく黄化しているが細胞が死滅しない限りは硝酸銀反応が全く負となる事はなく、正負の判定が困難な程微弱になっても尙生死両細胞を対比する時は両者の間に明か

な差を認めることができる。
クロマトグラフィーによる還元性物質の追求：
同生体組織における硝酸銀反応を惹起する還元要因はアスコルビン酸の局在であると多くの研究は一致した結果を示している。しかし尙植物細胞においてはこの反応をアスコルビン酸の組織化学的検出法として用いる場合にはより慎重な検討を望む者もあり、二三沈水植物についての上記の検討結果はこれ迄報告された陸上植物の場合と若干の相異点が見られる。それでこれらの植物についてクロマトグラフィーにより硝酸銀還元物質の検知を試みた結果は第1図の如くであって、アンモニア性、中性、酸性の各硝酸銀試薬により徐々にではあるが何れも顕著なクロマトグラムが現われ、夫々 Rf 約 0.08 附近に黒色~暗灰色のスポットを現わす。*Ceratophyllum* の場合だけ少しく上って Rf 約 0.1 位である。更に *E. densa*, *Hydrilla*, *Potamogeton* では前者に一部重複して Rf 約 0.13~0.14 附近に長楕円形のもう一つのスポットをも現はし、*Potamogeton* では他の二種類に比して小さい。又このスポットは同一種においても場合により大小、強弱を現わすことがあるが、Rf 0.08 の主たるスポットは殆んど一定共通であった。生育条件不良のため還元能の低下している



Fig. 1. Paper chromatograms (somewhat schematified) of the metaphosphoric acid extract from *Elodea densa* (1), *E. canadensis* (2), *Hydrilla verticillata* (3), *Potamogeton crispus* (4), and *Ceratophyllum demersum* (5), and of the metaphosphoric acid solution only (6), pure ascorbic acid solution with metaphosphoric acid (7), and pure glucose solution (8), developed with butanol-acetic acid mixture, and sprayed with neutral silver reagent (1~7) and benzidin reagent (8). The spots represent unknown new reducers (x, y), metaphosphoric acid (m), ascorbic acid (As), glucose (Gl), and originated from the solvent (s), respectively.

草体及び 30 日間暗培養されて殆んど還元能を失いかけている材料についてのクロマトグラムは Rf 0.13 のスポットが極めて微弱に見られるだけで、Rf 0.08 の主たるスポットは殆んど判定に困難か或は検出せられず、顕鏡的試験とクロマトグ

ラフィーの結果とはよく一致し、このスポットを生ずる物質が細胞内における還元剤中に高濃度のないことを証明している。すべての場合に停止線に達する細長い或は少しく溢れた極めて微弱なスポットも現われるがこれは盲検により溶剤に由来する微量混在物によるものであることが分った。これら被検液の調製には 5% メタ磷酸水溶液を用いたが、D.W.のみを抽出液として調製した場合にはこれらのスポットは全く現われないか現われても極めて微弱であって、安定剤としてメタ磷酸の存在を必要とするものであることを示した。メタ磷酸自体も硝酸銀試薬に反応する性質があるがメタ磷酸だけをクロマトグラフィーにかけた場合は Rf 0.24 の楕円形暗灰色のスポットと Rf 0.11 附近に不明瞭な暗部を現わすのみであってメタ磷酸は只還元性物質の安定剤として働くだけ

で Rf 0.8 及び 1.3 のスポットがメタ磷酸によるものでないことは明かである。この場合メタ磷酸自体のスポットの現れない理由は明らかではないが何らかの形で還元性物質と結合してそれを安定化しているのかも知れない。

これらのスポットが何であるかを示してアスコルビン酸であるかどうかをその特異反応及び純標品試験と対照した。アスコルビン酸により特異的に還元されて青色から無色になる 2,6-dichlorophenolindophenol 液を顕色剤とした場合停止線に近い溶剤由来のスポットが微紅色を現わした以外青地に他の変化は現われない。純アスコルビン酸 5 mg/cc D.W. 溶液のクロマトグラムは夫々硝酸銀液で黒褐色、Indophenol 液で青地に白色の Rf 0.4 の間かなるスポットを現わした。純アスコルビン酸の 5% メタ磷酸溶液での試験は Rf 0.375 の所に同様のより著しいスポットと Rf 0.25 の所にメタ磷酸のスポットを現わした。メタ磷酸の存在はアスコルビン酸の Rf 値を低下せしめ自体の Rf 値は若干上っている。これはアスコルビン酸が単に抽出液における硝酸銀還元スポットがアスコルビン酸でないということは確実である。

永井 (1951)⁵⁾によれば種によっては 3,4-dioxyphenylalanine (DOPA) が還元にあずかっている事もあり、それは potassium ferricyanide 等によっても顕色されるという。しかし報告せられた DOPA のクロマトグラム上の位置は此等草体抽出液に於ける位置と異なる。これは各イオンが離れた位置にあり、実際 potassium ferricyanide の顕色試験もこの位置に於いて反応しなかった。タンニン検出試薬として用いられる ferric chloride でも全く反応を示さずタンニンの存在も無いと思われる。還元糖検出のための Partridge-Westall のアンモニア性硝酸銀試薬により Rf 0.08, 0.13 のスポットが暗灰褐色に現われる。しかし Horrock, Manning (1949) の Benzidin 試薬の顕色に於て黄褐色の背景に暗褐色のスポットの特異的呈色像は認められず、却って Rf 0.08 の部分が黄褐色の背景に少しく空白のまま残される。Glucose 純標品のクロマトグラフィーの Benzidin 顕色は Rf 0.15 の著しい特異的呈

色像が現われ、これは草体抽出液による Rf 0.13 の硝酸銀還元スポットと位置的に近似してはいるがそれ自身は Benzidin 顕色に全く現われないのであるから此等のスポットが同一でないことも明らかである。

考 察

永井 (1950)⁴⁾は陸生高等植物について詳細な研究をなし典型的な Molisch 反応は酸性試薬に於いて最も著しく、アンモニア性又は液胞、細胞質に一樣黒色綿毛状沈殿を顕著に生ずるが葉緑体の着色は極めて弱く不規則に生ずる。完全なる暗黒処理に於て葉緑体の着色は全く起らず、僅かな沈殿が不規則に生ずるにすぎない事を報告し、同様の結果は Metzner (1952)⁸⁾によっても報告されている。それに比べてここで取上げられた沈水植物細胞では若干反応の様相に相異点があり、単に細胞内に生ずる還元銀量だけを見るならばやはりアンモニア性試薬に於て最も著しいが、葉緑体の特異的黒化即ち所謂 Molisch 反応の生起に注目するならば、単なる試薬浸漬処理でも葉緑体の着色は幾分か認められるがその程度は弱く、アンモニア性試薬に於て最も著しい。更に、酸性及び中性試薬による加熱処理に於て最も典型的であり概して

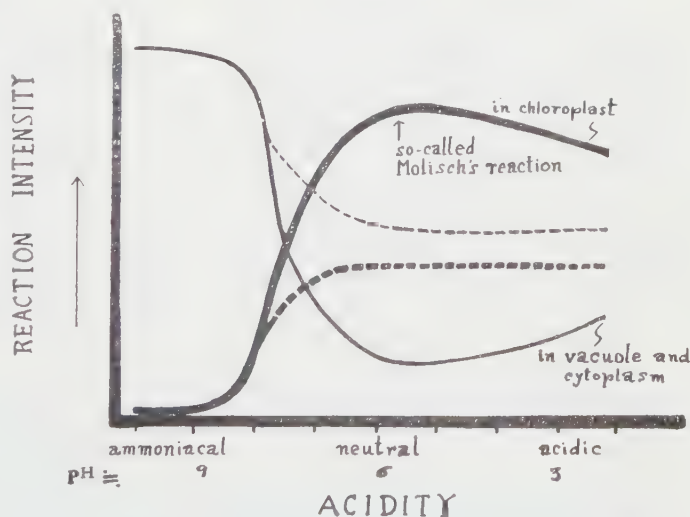


Fig. 2. Experimental diagram showing the relation between reaction intensity in chloroplast or in vacuole and cytoplasm and acidity of silver reagent.

—: Heat treatment.

- - -: Soaking treatment only.

中性において優っているようである。(Fig. 2) 永井・尾形 (1952)¹⁷⁾ は又海藻では酸性より中性においてより好適な特異的結果をうる事を見ているが此等の場合もむしろそれに近い傾向が認められる。何れの場合にも処理時における明暗条件は反応に殆んど差異を生じておらず、Gautheret (1938)⁹⁾, Mirimanoff (1938)¹⁰⁾ 等も指適しているような光の不可欠性はこの場合必ずしも認められない。

所謂 Molisch 反応は葉緑体における還元性物質の局在によるとの従来の定説に対し、永井 (1950)⁷⁾ はその反応生起の機構について種々の実験結果から還元性物質はむしろ液胞及び細胞質内に分散しているのであって、葉緑体の黒化は還元銀膠質粒子が二次的条件によって吸着されて起るものであると説明し、pH が大きく反応生起に影響するところから荷電関係による吸着を考えている。Caruso (1938)¹¹⁾, Savelli and Caruso (1938, 1939)¹²⁾, Danielli (1953)¹³⁾ 等も還元性物質の葉緑体への局在を疑問視し反応生起には他にも要因がある事を示唆している。Höfler (1939)¹⁴⁾ も孔辺細胞に於て条件により速かに一様分散型、葉緑体特異型が現われる事を見ている。此等沈水植物細胞に於ても試薬の酸性度により夫々前記の様に反応の様相を異にし、アンモニア性では一様に反応が強く起るのに酸性に傾くにつれ葉緑体に顕著となるのは此等も同様に酸性度に支配される二次的吸着により葉緑体反応が生起するものであるが、単なる試薬浸漬ではむしろ液胞、細胞質中の一様暗化、綿毛状沈殿の方が著しく、加熱処理によって始めて強い葉緑体の特異的黒化が見られるのは、陸生植物に比べて此等の吸着関係が弱く加熱処理がそれを飛躍的に拡大するために葉緑体の特異的黒化をもたらすもので、必ずしも葉緑体における還元性物質の局在を意味するものではなく、還元性物質それ自体はむしろ液胞及び細胞質中に一様に分散して存在しているものと考えられる (Fig. 2)。

Molisch 反応の強さとでんぶん量との関係について Höfler (1939)¹³⁾ は密接に関連する事を示し、永井 (1950¹⁵⁾, 1953¹⁶⁾ は直接的関係は認められないと言い又黄化した芽生え即ち葉緑素のないものでは反応は生じない事を報告している—永井・尾形 (1952)¹⁷⁾。ここではでんぶん量

及び葉緑素量と大凡の平行関係が認められたが必ずしも絶対的なものでなく、常に平行とは限らない。これは反応の強さがむしろ細胞及び葉緑体の vital activity に、より密接な連関性をもっている事を示唆するもので、でんぶん、葉緑素量即ち光合成能との平行は間接的なものであろう。Molisch (1918)³⁾, Geitler (1922)¹⁸⁾ 等は既にこの反応が細胞の生死判別に利用できる事を注目し、尾形・永井 (1953¹⁹⁾, 1954²⁰⁾ は藻体細胞の生死、被害判定等に此が有効な実用的手段である事を示した。ここでもその事実は明かに認められ、vital activity の低下に平行して反応の強さは減弱したが細胞が生きている限りはどんなに微弱であっても生死両細胞を比較する時は明かに差異を認めることができる。原形質分離細胞が正常細胞と全く同じ反応を示す事は既に永井 (1950)⁹⁾ も報告しているがここでも単なる原形質分離そのものによる細胞の vital activity の低下は少いものである事を示している。

Szent-Györgyi (1928)¹⁾ 以来 Giroud-Leblond (1934)²¹⁾, Bourne (1936)²²⁾, Weier (1938)²³⁾ 等その他多くの研究により硝酸銀還元物質はアスコルビン酸であることが決定せられ、永井・尾形 (1950⁷⁾, '51⁵⁾, '52¹⁷⁾, '53¹⁶⁾ は更にクロマトグラフィーにより詳細広範な研究をなし、陸生高等植物のみならず海藻類においてもアスコルビン酸が共通の還元要素である事を明かにした。しかし同一の手法に従ってなされた此等沈水植物での結果は全く異なつた結果を示し、現われた硝酸銀還元スポットは被験種に略共通であったが予想に反してアスコルビン酸のスポットとは全く異り、更に DOPA でもなくタンニン系物質でもなく又還元糖でもなかった。*in vitro* でのモデル実験の結果はアスコルビン酸はアンモニア性試薬を最も強く且つ敏感に還元することを示し、永井 (1951)⁵⁾ もクロマトグラフィーでアンモニア性試薬がインドフェノール試薬について強くアスコルビン酸を検出し得る事を報告し、又アスコルビン酸以外にもフラボノイド、タンニン、還元糖等種々のスポットが現われる事もあったが、これらはすべてアンモニア性試薬でのみ検出され、酸性試薬では反応しなかったという。それに対しこの場合はアンモニア性、中性、酸性のすべての試薬に反応し陸生高等植物及び海藻類等におけるものとは異つた

別の物質であると思われる。Rf 0.08 及び 0.13 の此等の物質は何れも分解乃至は酸化を容易い不安定な物質でもって、抽出に際しては安定剤としてメタ燐酸の存在を必要とする事はアスコルビン酸に類似しているが還元力は比較的に弱く鈍い。Rf 0.13 のスポットは *E. canadensis*, *Ceratophyllum* では現われず、又場合によりかなり消長があって不安定な還元力も弱く、此の意義は従属的なものであると思われる。此等二つのスポットが物質的に何であるかは尙決定できなかったが、此等沈水植物は陸生植物に比し若干の点で相異が認められるが大凡類似の著しい還元反応を示すにも拘らず還元物質の分画は全く違った物質の存在を示した事は注目に値しよう。

Giroud-Leblond 法はアスコルビン酸の細胞化学的検出法としては必ずしもその特異性及び敏感度が十分でない事は既に Mirimanoff (1938)²¹⁾, 新家・重永 (1947)²²⁾, 飯島・平岡 (1950)²³⁾, 及び Danielli (1953)¹³⁾ 等によって注意され、又種によっては蔭酸の存在が著しく反応に干渉する事が永井・尾形 (1952)¹⁷⁾ によって明かにされた。沈水植物においては上記の実験結果がアスコルビン酸以外の物質によった事を明かにし、又試薬の pH, 加熱等の二次的條件による反応生成物の著しい易動性はこの処理法が細胞化学的検出法としてすべての場合に絶對的にアスコルビン酸の存在及びその局在性を示すものとは言いかねず、又結果の判定には尙より以上に慎重な考慮が必要とされるであろう。

傷害を受け、死滅した細胞での還元反応の消失は極めて鋭敏であって生死両細胞の対照は全く顯著であるが、Weier (1938)²³⁾ によればこれは還元性物質が酸化されて還元力を失うからであって、酸化を防止する条件下では死細胞でも反応の

生起を認め得たと言う。この場合安定剤としてメタ燐酸が存在すればこの還元性物質は磨砕抽出によってもクロマトグラフィーにおいて充分強い還元力が保持されていたが、メタ燐酸液中において切断された細胞でも顕微鏡下では完全に反応能を失い、又単にメタ燐酸液中に浸漬せられただけでもそれによって害をうけ原形質は不規則に収縮し、硝酸銀反応は負となった細胞外に磨砕抽出せられる場合にすらメタ燐酸は充分有効な安定剤であるのだから、たとえ傷害死滅細胞内においてもこの場合還元性物質は安定化されている筈で、葉緑体の特異的黒化はともかくとしても少くとも一次的な硝酸銀還元は起ってよいと思われるのに顕微鏡下において全く何らの反応も認められないのは奇妙である。*in vivo* においては“生きている”という何らかの微妙な条件が必要なのであるか、或は又還元性物質自体はメタ燐酸により安定化されてはいても死滅細胞には反応を阻止する条件が出現するのであろうかとも考えられる。*E. canadensis* のメタ燐酸磨砕遠心上澄液中に中性硝酸銀液を滴下したところ著しく白濁し、帯淡黄褐色の沈殿を生じ、先にアスコルビン酸での *in vitro* のモデル試験において見られたような還元銀の白色沈殿は認められなかった。この状況は永井・尾形 (1952)¹⁷⁾ が反応干渉物質の研究で報告している状況と類似している。クロマトグラフィーで展開されれば各物質が分離され、安定化された還元性物質は他に妨げられることなく硝酸銀試薬により暗灰色のスポットを生ずるのかも知れない。しかし此等の関係を説明するためには尙将来の検討にまたねばならない。

終りに有益な御助言を下された本学教育学部相馬倂介教授、東京教育大学植田利喜造助教授に謝意を表する。

Summary

Several studies on the silver-nitrate reduction in the cells of five species of the submerged plants were carried out.

1. The reduction occurred as in the land plants, but the specific blackening of chloroplast (so-called “Molisch’s reaction”) was most typical when the material was heated with neutral silver reagent.

2. The light and dark conditions in procedure had no influence on the occurrence of reduction.

3. The specific blackening of chloroplast does not always indicate the localization of the reducing agent in it. It is due to the secondary deposition of reduced silver on chloroplast, and the agent is rather contained diffusely through the vacuole and cytoplasm.

4. The parallelism between the reaction intensity of chloroplast and the starch and chlorophyll contents was not always absolute, but it was connected rather more closely with the vital activity of cell and chloroplast.

5. The trials of detection of reducing substances by means of the chromatography showed the silver reducing spots in the position of R_f 0.08 in all cases and also of 0.13 in a few cases. These spots were not ascorbic acid, DOPA, reducing sugar, tannin nor flavonoid etc. For the extraction of these substances the presence of metaphosphoric acid was necessarily required as a stabilizer. The identification of these substances is as yet remained unsettled.

6. It must be considered, from these facts, that the Giroud-Leblond's procedure or silver-nitrate reagent are not always sufficiently specific in all cases for ascorbic acid in the cytochemical use.

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帽菌類の diploidisation における核の行動

III. 和合性組合わせの場合*

木 村 勘 二**

Katsuji KIMURA**: Nuclear Behaviour in the Diploidisation of the Hymenomycetous Fungi III. Legitimate Combinations*

昭和32年9月9日受付

Buller (1931) は *Coprinus lagopus* (四極性) を用いての diploidisation の研究の結果, $AB \times (AB+ab)$ のような和合性組合わせでは接種した複相菌糸の2核の中, ab 核だけが AB 大菌叢中を核分裂をしながら移行するものであろうと述べており, この説は Quintanilha (1933, 1939) 等の支持を得た。一方, Dickson (1934~1936) は *Coprinus sphaerosporus* (二極性), *C. macrorhizus* (四極性) で diploidisation の実験を行い, ある菌叢を印刷につけた菌糸を用いての和合性組合わせで, 接種した複相菌糸の2核がともに大菌叢の中を移行したと思われる結果を数例得ており, Quintanilha (1939) も *C. fimetarius* (四極性) を用いての和合性組合わせの実験で, 同様の結果を1例得ている。著者 (1957a) は不和合性組合わせの実験の中で, 和合性組合わせにおいても2核が移行するという実験例を二つ得た。

著者 (1957 a, b, 1958) は前に不和合性, 両和合性のどちらの組合わせにおいても2核は移行するものであることを証明したが, 上述の著者から推して, 和合性組合わせにおいてもまた, 2核は移行するものと思われる。しかし, これらの2核移行を示す結果は, いずれも偶然に得られたものもあり, その数も非常に少く, 和合性組合わせにおける2核移行を証明するには少くも不充分とも考えられる。そこで著者は和合性組合わせで, 2核移行を示す結果が多数得られるように, 用いる菌糸に考慮をめぐらして実験したところ, すなわち目的を達し得たと思われるから, ここに, その究めたところを報告する。

材 料

本実験にはウシグソヒトヨ *Coprinus macrorhizus* Rea f. *microsporus* Hongo (四極性) の V, X, Y, c, d, e の6系統を供試した。これら各系統の産地, 不和合性因子等については既報(木村 1957a) を参照されたい。

実験並びに結果とその考察

一つの系統だけを用いて和合性組合わせ $AB \times (AB+ab)$ を行つては, 大菌叢の周縁部に現われた複相菌糸が $AB+ab$ であっても, その AB 核は接種した複相菌糸から移行して来たものか, あるいは原菌糸自体のものかの区別がつかない。もとより, Dickson の場合のように, どちらかの AB 核を突然変異によって生じたある形質で標識しておけば, その区別がつかないが, 著者の場合にはそのような菌糸を得ることができなかったから, 不和合性因子を異にする2系統を組合わして実験した。すなわち, $AaBb$ と $A'a'B'b'$ の2系統の AB 核と $A'B'$ 核とが和合して生じた子実体から単胞子培養を行い, \underline{AB} , $\underline{A'B'}$, $\underline{AB'}$, $\underline{A'B}$ の四つの交配型の菌糸を得た。同様に ab 核と $A'B'$ 核とが和合して生じた子実体から \underline{ab} , $\underline{A'B'}$, $\underline{aB'}$, $\underline{A'b}$ の交配型の菌糸を得た。これらの新菌糸と $AaBb$ 系統の原菌糸 AB , ab , Ab , aB を用いて, 2核移行を示す結果が得られることが予想されるような和合性組合わせを試みた。

第1表の組合わせがそれで No. 1~16 は, みな次のような条件に於けるものである。すなわ

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** 岡山大学理学部生物学教室 Department of Biology, Faculty of Science, Okayama University, Okayama, Japan.

第1表 和合性組合わせによる diploidisation (1)

番号	組合わせ	供2 系統 試統	出現複相菌系の核構成				
			c, X	d, X	d, e	X, V	X, Y
1	$AB \times (A'B' + Ab)$		f, f	d, d	○	×	×
2	$AB \times (A'B' + aB)$		f	/	○	×	×
3	$AB' \times (A'B + Ab)$		/	×, ×	×	×	○
4	$A'B \times (AB' + aB)$		○○, ○○○	×, ○*	◎	×	×
5	$AB \times (ab + AB')$		×	×	×	○	×
6	$AB \times (ab + A'B)$		×	×	×	○	○
7	$Ab \times (aB + AB')$		×	/	×	○	○
8	$aB \times (Ab + A'B)$		○	/	×	○	○
9	$ab \times (A'B' + Ab)$		×	/	×, ×	○	○+×
10	$ab \times (A'B' + aB)$		×	/	×, ×	×	○○○
11	$aB' \times (A'b + aB)$		s	/	×, ×	×	○
12	$A'b \times (aB' + Ab)$		○	/	×, ×	×	○
13	$ab \times (AB + aB')$		○	/	×, ×	○	○
14	$ab \times (AB + A'b)$		×	/	×, ×	×	○○○
15	$Ab \times (aB + A'b)$		×	×	×, ×	×	○
16	$aB \times (Ab + aB')$		×	×	×, ×	*	×××
17	$AB' \times (ab + AB)$		/	/	×	×	○○*
18	$A'B \times (ab + AB)$		×, ×, ×	×	×	×	×××
19	$AB' \times (A'b + AB)$		×	/	×	×	○
20	$A'B \times (aB' + AB)$		s, s, s	×	×	×	×
21	$aB' \times (AB + ab)$		s, s, s	×	○	×	○
22	$A'b \times (AB + ab)$		×	s	×	×	○
23	$aB' \times (A'B + ab)$		s, s, s	×	×	×	×
24	$A'b \times (AB' + ab)$		×	○	×	×	○

○印は接種した複相菌系の2核が和合し、2核移行を証明する結果が得られた場合

×印は大菌叢の核と、接種した複相菌系の中の和合性の核とが和合した場合

◎印は大菌叢の核と、接種した複相菌系の中の不和合性の核とで複相菌系を作った場合

*印は接種した複相菌系の2核の染色体交換によって生じた新核が大菌叢の核と和合した場合

dは組合わせをしても大菌叢の周縁部に複相菌系が現われなかった場合

fは出現複相菌系を培養しても子実体が生じなかった場合

sは子実体が生じても胞子ができなかった場合

/印は組合わせを行わなかった場合

同一周縁部に現われた複相菌系を2ヶ所以上からとって分析した場合は、その結果を列記してアンダーラインを引いた。同一の組合わせを同時に2回以上行った場合は、各回の結果の間にコンマを入れた。供試2系統の欄で、先に記した系統の不和合性因子を $AaBb$ 、後に記した系統のそれを $A'a'B'b'$ とした。

ち、いずれの組合わせにおいても、接種した複相菌系の2核の中、大菌叢の核と和合できる方の核は大菌叢の核と同じ子実体から由来したものであり、いま一つの和合できない方の核は大菌叢の核とは異なる子実体から生じたものである。

著者は既報(1957 b, 1958)で本菌の2核の和合には A, B の不和合性因子の外に、和合の強さを左右する変異因子も関係することを仮定し、和合できる2核の間の変異因子の差が大きい程、和合性が強く、また両和合性組合わせによる diploi-

disation においては、接種した複相菌糸の 2 核の中、大菌叢の核と変異因子の差が大きい方が早く大菌叢の中を移行することを推論した。

もし、この仮説が正しいならば、No. 1~16 の組み合わせでは次のことが期待される。例えば No. 1 の $\underline{AB} \times (\underline{A'B'} + \underline{Ab})$ では、 \underline{AB} 核と $\underline{A'B'}$ 核は原菌糸の \underline{AB} , $\underline{A'B'}$ の 2 核が融合、減数分裂して生じたものであるから、既報 (木村 1957 b, 1958) のように両者の変異因子の間に共通性が存在し、従って \underline{AB} , $\underline{A'B'}$ 間の変異因子の差は、 \underline{AB} , \underline{Ab} 間又は $\underline{A'B'}$, \underline{Ab} 間のそれよりも小さいと一応考えられる。故に、もしこの組み合わせで大菌叢の \underline{AB} 核と和合できる $\underline{A'B'}$ 核は勿論のこと、 \underline{AB} 核と和合できない \underline{Ab} 核までも大菌叢の中を移行するものがあるならば、 \underline{AB} 核に対して $\underline{A'B'}$ 核よりも変異因子の差の大きい \underline{Ab} 核の方が早く進んで \underline{AB} 菌糸の先端に達するであろう。しかし \underline{AB} , \underline{Ab} の両核は和合できないから、2 核は複相菌糸を作らずに遅れて到着する $\underline{A'B'}$ 核を待ち $\underline{A'B'}$ 核が菌糸の先端細胞に達したら \underline{AB} , \underline{Ab} , $\underline{A'B'}$ の中の 2 核が和合して初めて複相菌糸が生ずるが、この場合、変異因子の差が大きいと思われる \underline{Ab} と $\underline{A'B'}$ の 2 核が大菌叢の \underline{AB} 核をさしおいて和合するであろうから、和合性組み合わせにおける 2 核移行を証明する複相菌糸の出現に期待がもてる。

しかし、この期待に対して不利な条件が一つある。既報 (木村 1957 b, 第 3 表) の両和合性組み合わせ、例えば $\underline{AB} \times (\underline{ab} + \underline{A'B'})$ では \underline{AB} , \underline{ab} 間の変異因子の差の方が \underline{AB} , $\underline{A'B'}$ 間のそれよりも大きい場合が多く、従って \underline{ab} 核の方が早く進む場合が多いと論じたが、各々の和合性組み合わせ、例えば $\underline{AB} \times (\underline{A'B'} + \underline{Ab})$ では \underline{Ab} 核の方が $\underline{A'B'}$ 核よりも早く進む場合が多いというわけにはいかない。何となれば、 \underline{Ab} 核と \underline{AB} 核とは由来した子実体は異なるが、不和合性因子の \underline{A} に関しては共通であるから、変異因子の若干が \underline{A} 因子と連鎖している場合は、それらの変異因子に関しては \underline{Ab} , \underline{AB} の両核は同一とはいえぬまでも、両者の間に常に共通性が存在するものと考えられる。従って \underline{Ab} , \underline{AB} 両核間の変異因子の差は、このハンディキャップによって、上述の両和合性の場合の \underline{ab} , \underline{AB} 間の差ほど大きくない場合が多いから、 \underline{Ab} , \underline{AB} 間の差の方が \underline{AB} , $\underline{A'B'}$

間の差よりも大きい場合があり得るという程度になるであろう。

第 1 表の No. 17~24 の和合性組み合わせは No. 1~16 のそれとは少し趣きを異にし、例えば No. 17 の $\underline{AB'} \times (\underline{ab} + \underline{AB})$ では $\underline{AB'}$, \underline{ab} の 2 核は互いに由来した子実体を異にする。しかし、どちらも原菌糸の $\underline{A'B'}$ 核を両親の一つにもつから、両者の間に共通な変異因子が存在することが考えられるが、両親の他の一つが互いに異なるから、この共通性も No. 1 の組み合わせの \underline{AB} , $\underline{A'B'}$ 間のそれに較べると概して小さいであろう。従って、この組み合わせにおいて \underline{AB} 核の方が \underline{ab} 核よりも早く進む可能性は一層減ずるものと思われる。

以上のような予想の下に実験を行い、各組み合わせに現われた複相菌糸の 2 核を分析した。分析の方法は既報 (木村 1954 a, b) のように出現複相菌糸の一部をとって培養し、生じた子実体から分離した単胞子培養菌糸の交配型を調べて、その結果から出現複相菌糸の核構成を推定した。なお、若干の組み合わせについては同時に 2 回以上行い、或いは又、同一周縁部に現われた複相菌糸を 2 ケ所以上からとり、その核構成を分析した。培養基、培養温度等は既報に準ずる。

以上の実験の結果は第 1 表に示されるように、供試した 2 系統によって 2 核移行を示す結果の出現率は異なるが、核構成を分析したものを合計すると、2 核移行を示したものは組み合わせ No. 1~16 では 66 の中の 24 (36.4%)、No. 17~24 では 33 の中の 7 (21.2%) であった。この結果は、まず期待に近いものと考えられ、これらの多くの例証から和合性組み合わせにおいても接種した複相菌糸の 2 核は大菌叢中を移行するものであるといえるであろう。

大菌叢の周縁部の 1 ケ所に複相菌糸を接種してから 2 核移行を示す複相菌糸が出現するまでの核の行動は、著者が初め想像したようなものであったかどうかを検するため更に次の実験を行った。

第 1 表で X と Y の 2 系統を用いた実験の中、No. 14 の組み合わせ $\underline{ab} \times (\underline{AB} + \underline{A'b})$ の周縁部に現われた複相菌糸は 3 ケ所共に等しく $\underline{AB} + \underline{A'b}$ で 2 核移行を示し、No. 17 の組み合わせ $\underline{AB'} \times (\underline{ab} + \underline{AB})$ の周縁部の複相菌糸は、2 ケ所は $\underline{ab} + \underline{AB}$ で 2 核移行を、他の 1 ケ所は $\underline{AB'} + \underline{aB}$ であって \underline{ab} , \underline{AB} 両核の間の染色体交換によって

生じたと思われる ab 核が AB' 核と和合したことを示した。第 1 表にわかるように、同様な新核出現は d, X 2 系統供試の No. 4 の組合わせ、及び X, V 2 系統供試の No. 16 の組合わせにおいても見られた。このような新核出現は不和合性組合わせでは Quntanilha (1938), Papazian (1950) 及び著者 (1957 a) が見ているが、両和合性組合わせでは Papazian がその実験例を得ているだけであり、和合性組合わせにおいては著者の知る範囲では今度の例が最初のものと思われる。

上記の No. 14 及び No. 17 の組合わせを再び行い、各々に出現した複相菌糸を数カ所からとって既報 (木村 1958) のように単菌糸分離を行った。既報の両和合性組合わせに現われた複相菌糸の単菌糸分離の場合には、分離後生長した菌糸は全部 clamp を有する複相菌糸であったが、今度の和合性組合わせの場合は分離後生長した菌糸は複相のものの中に、単相の状態のものもあった。

先ず No. 14 の組合わせ $ab \times (AB + \underline{Ab})$ から単菌糸分離した複相菌糸 5 個の核構成を分析したところ、5 個共等しく $AB + A'b$ であって $ab +$ は 1 個もなく、周縁部の複相菌糸の全部が移行 2 核で構成されたものと思われた。次に同じ菌叢周縁部から分離した単相菌糸 10 個を標準菌糸と組合わせ培養して交配型分析した結果は次の通りである。

		1	2	3	4	5	6	7	8	9	10
X 標準 系菌 統糸	AB	+	+	+	+	+	+	+	+	+	+
	ab	-	-	-	-	-	-	-	-	-	-
	Ab	-	-	-	-	-	-	-	-	-	-
	aB	+	-	+	+	-	+	+	-	+	+

この結果から上記単相菌糸の中、No. 2, 5, 8 は接種した複相菌糸の 2 核のいずれも、まだ移行して来ておらぬ純粋な ab 菌糸であり、No. 1, 3, 4, 6, 7, 9, 10 は自体の ab 核の外に、接種した複相菌糸の 2 核の中、早く進んで来た $A'b$ 核もはいっているが、この 2 種類の核は互いに不和合のために単相菌糸の状態に止まっているものであると考えられる。そして接種した複相菌糸の他の核 AB が遅れて到達した場合、菌糸端の細胞中に存在するこれら ab , $A'b$, AB の 3 核の中では和合性が ab , AB 間よりも強い $A'b$, AB の 2 核の間で和合が行われ複相菌糸が生じていくが、こ

のようなことはどの菌糸端の細胞においても同じように行われることは、前述の周縁部の複相菌糸の核構成が等しく $AB + A'b$ であったことが証明する。故にこれらの和合性組合わせにおける核の行動は全く著者の予想通りであったといえよう。

次に No. 17 の組合わせ $AB' \times (ab + AB)$ より分離した複相菌糸 5 個の 2 核を分析した結果は、 $ab + AB$ が 1, $AB' + ab$ が 2, $AB' + aB$ が 2 であり、同一の菌叢の周縁部に 3 種類の複相菌糸が現われたことを示した。恐らく、この組合わせでは AB' , ab , AB の 3 核相互間の変更因子の差が僅少であるため、 ab , AB の 2 核は殆んど同じ早さで進み、ある AB' 菌糸の中では ab 核が先行して AB' 核と和合し $AB' + ab$ 菌糸を作り、他の AB' 菌糸では AB 核が先行して、後で ab 核が到着してから、 $AB + ab$ 又は $AB' + ab$ 菌糸が生ずるものであろう。また、この場合、先端細胞中の 3 核間の変更因子の差が少いため、どの 2 核が結びつくかということに混乱が生ずることも考えられ、この時に ab , AB 2 核の間に染色体の交換が起って新核 aB , Ab が生じ、この中の aB 核が AB' 核と和合した結果、 $AB' + aB$ 菌糸も出てきたのではあるまいか。このようなことは第 1 表の d, X 2 系統供試の組合わせ No. 4 の結果からも想像される。上記のように組合わせ No. 17 においては $ab + AB$, $AB' + ab$, $AB' + aB$ の 3 種類の複相菌糸が現われるのに第 1 表では $AB' + ab$ 菌糸が見られなかった。これは偶然にこの菌糸の存在する部分をとらなかったためとも思われるが、また次のようにも考えられる。出現複相菌糸の核構成は、その複相菌糸に生じた子実体から分離した単胞子培養菌糸を交配型分析して決めているのであるから、 $AB' + ab$ 菌糸が混在していても、既報 (木村 1958) で述べたような複相菌糸間の子実体形成能力の優劣が原因として、結果には出なかったのではなからうか。何となれば $ab + AB$, $AB' + ab$ の 2 種類の複相菌糸の混合培養を同時に平行して 5 回行ったところ、 $ab + AB$ の子実体だけが生じたからである。

次に変更因子の構成によって移行する核の早さが左右されることの証明の一つとして、次の実験を行った。第 1 表の X, Y 2 系統供試の組合わせ No. 17~24 で、原菌糸の AB , ab の代りに新菌糸の AB , ab を使って、再び同じ組合わせの実験

を行った(第2表)。この場合、AB 核は No. 17' ~20', また ab 核は No. 21' ~24' の各組合わせにおける単相大菌叢の核と由来した子実体を一にするからAB 核や ab 核が先行する可能性は早急に減ずるものと思われる。この実験の結果は第2表のようであるが、2核移行を示したものは僅かに1例で、第1表の5例に較べて少く、予想通りであったといえよう。

第2表 和合性組合わせによる
diploidisation (2)

番号	組 合 わ せ (X, Y 供試)	出現複相菌糸の核構成
17'	<u>AB'</u> × (<u>ab</u> + <u>AB</u>)	<u>AB'</u> + <u>ab</u> ×
18'	<u>A'B</u> × (<u>ab</u> + <u>AB</u>)	<u>A'B</u> + <u>ab</u> ×
19'	<u>AB'</u> × (<u>A'b</u> + <u>AB</u>)	<u>AB'</u> + <u>A'b</u> ×
20'	<u>A'B</u> × (<u>aB'</u> + <u>AB</u>)	<u>A'B</u> + <u>aB'</u> ×
21'	<u>aB'</u> × (<u>AB</u> + <u>ab</u>)	<u>aB'</u> + <u>AB</u> ×
22'	<u>A'b</u> × (<u>AB</u> + <u>ab</u>)	<u>A'b</u> + <u>AB</u> ×
23'	<u>aB'</u> × (<u>A'B</u> + <u>ab</u>)	<u>aB'</u> + <u>A'B</u> ×
24'	<u>A'b</u> × (<u>AB</u> + <u>ab</u>)	<u>AB'</u> × <u>ab</u> ○

C, ×印の意事は第1表に準ずる

第1表の d, e 2 系統供試の No. 4 の組合わせ

A'B × (AB' + aB) に現われた複相菌糸は正常な子実体を生じ、胞子の量, 形, 発芽率等もすべて正常であった。そして、この子実体よりの単孢子培養菌糸 25 個の交配型分析の結果は 16 個が aB, 9 個が A'B であったから、著者は出現複相菌糸の核構成は A'B + aB であると断じたが、不和合性因子 B が共通である 2 核が正常と思われる複相菌糸を作ったことは注意をひく。

また、第1表の X, Y 2 系統供試の No. 9 の組合わせ ab × (A'B' + Ab) に現われた複相菌糸は子実体を形成したが、胞子は僅少で形は異常、発芽率は非常に低かった。そして、これらからの単孢子培養菌糸の中には単相のものもあったが、clamp を持った複相のものも相当数見られた。単相のものの交配型分析の結果は、これらが A'B' + Ab の減数分裂によって生じたものであることを示した。一方、複相のものが形成した子実体から更に単孢子分離培養を行い、それらを交配型分析した結果は、この複相菌糸の核構成が ab + A'B' であることを示した。それ故、No. 9 に現われた複相菌糸が形成した子実体には ab, A'B', Ab の 3 核が関係していると考えられる。

以上述べた異常な二つの結果については現在、更に追究中であるから、これらの詳細については後日の報告にゆずることにする。

Summary

To prove the two-nuclear migration from the diploid inoculum into the large haploid mycelium in the diploidisation by the compatible diploid mycelium, the experiment was performed, using *Coprinus macrorrhizus* f. *microsporus*.

The legitimate combinations, e.g. AB' × (A'B + Ab) in which the nuclei AB' and A'B derived from the same fruit-body and the nucleus Ab derived from a different one, were made. In some of these combinations, the nucleus Ab advanced more rapidly than A'B; and, when the nucleus A'B arrived late at the terminal cell of the haplont AB', conjugation occurred between the nuclei Ab and A'B.

The above results may be explicable from the assumption that the speed of nuclear migration and the conjugation affinity between two compatible nuclei are controlled by the modifiers.

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側枝及び不定根と母軸との維管束連絡 トウモロコシの維管束解剖 第6報

熊 沢 正 夫*

Masao KUMAZAWA*: Vascular Connection of the Axillary Shoot and the Adventitious Root with Their Mother Axis. Vascular Anatomy in Maize. VI.

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緒 言

茎の基本組織中に多数の維管束が散在している単子葉類では、その維管束走向の正確な追及は至難であるため、従来この方面の真に信頼し得る研究は極めて少い。従って筆者はトウモロコシについて、その点の知見を第1報(1939)乃至第5報(1946)として報告して来た。然しこれ等の報告では雌花穂内の維管束走向及び腋芽と不定根との母軸に対する維管束連絡については触れることがなかった。雌花穂内の維管束走向については Laubengayer (1946, 1948, 1949) や Reeves (1946, 1950, 1953) の研究がその後出されたから、改めて筆者が検討する必要がなくなった。腋芽及び不定根と母軸との維管束連絡については第5報(1946)執筆当時既に大凡研究してあり、第5報で既に続報することを予告しておいた。然し戦禍によりその記録一切を失い続報を中断してあった。それでその後改めて研究し、本報で側枝母軸間及び不定根母軸間の維管束連絡の状況を略述し、次報を以って一応の結末をつけて責を果すことにする。

以下記述する所見は砂糖トウモロコシに属する Silver Bantam 又は Golden Bantam を材料とし、ミクロトームによる連続切片と徒手切片とを併用して得られたものである。

側枝と主軸との維管束連絡

Fig. 1, 1 の左半分は主桿、右半分はその葉腋から発達した側枝(果柄)基部である。この側枝の左側上下に近く、それぞれ2個の維管束が他のものから少し分離して位置するが、これは逆生前出葉(Inverted prophyll)から由来する葉跡条の1部である。この水準から初めて桿を順次下方へたどった横断面が Fig. 1, 2~7 であり、Fig. 1, 5 では該側枝の蓋葉の葉鞘基部が丁度桿に殆ど合着した水準を示し、斜線をつけた最上部の維管束は蓋葉から由来した葉跡条である。

Fig. 2, 1~2 は桿の節部附近における維管束走向の模式図で、側枝はその基部の断面を以て示しており、断面を黒く示した維管束は筆者の言う合成維管束(1942, p. 530), L は髓に深く入り込む蓋葉の葉跡条で、筆者の第I型(1940 b, p. 495)となすもの、1はこの節で直に母軸の最辺周部維管束(1940 a, p. 311)に合着し、第II型(1940 b, p. 502)と呼ぶ蓋葉からの小型葉跡条である。Fig. 2, 1 は母軸及び側枝のそれぞれ最辺周部維管束間、Fig. 2, 2 は同じく母軸及び側枝の合成維管束相互間の維管束連絡を示すが、共に節網維管束(1942, p. 528)は図示を省略してある。

今 Fig. 1, 1 における母軸の合成維管束の内、

* 名古屋大学教養部生物学教室 Biological Laboratory, Department of General Education, Nagoya University, Nagoya, Japan.

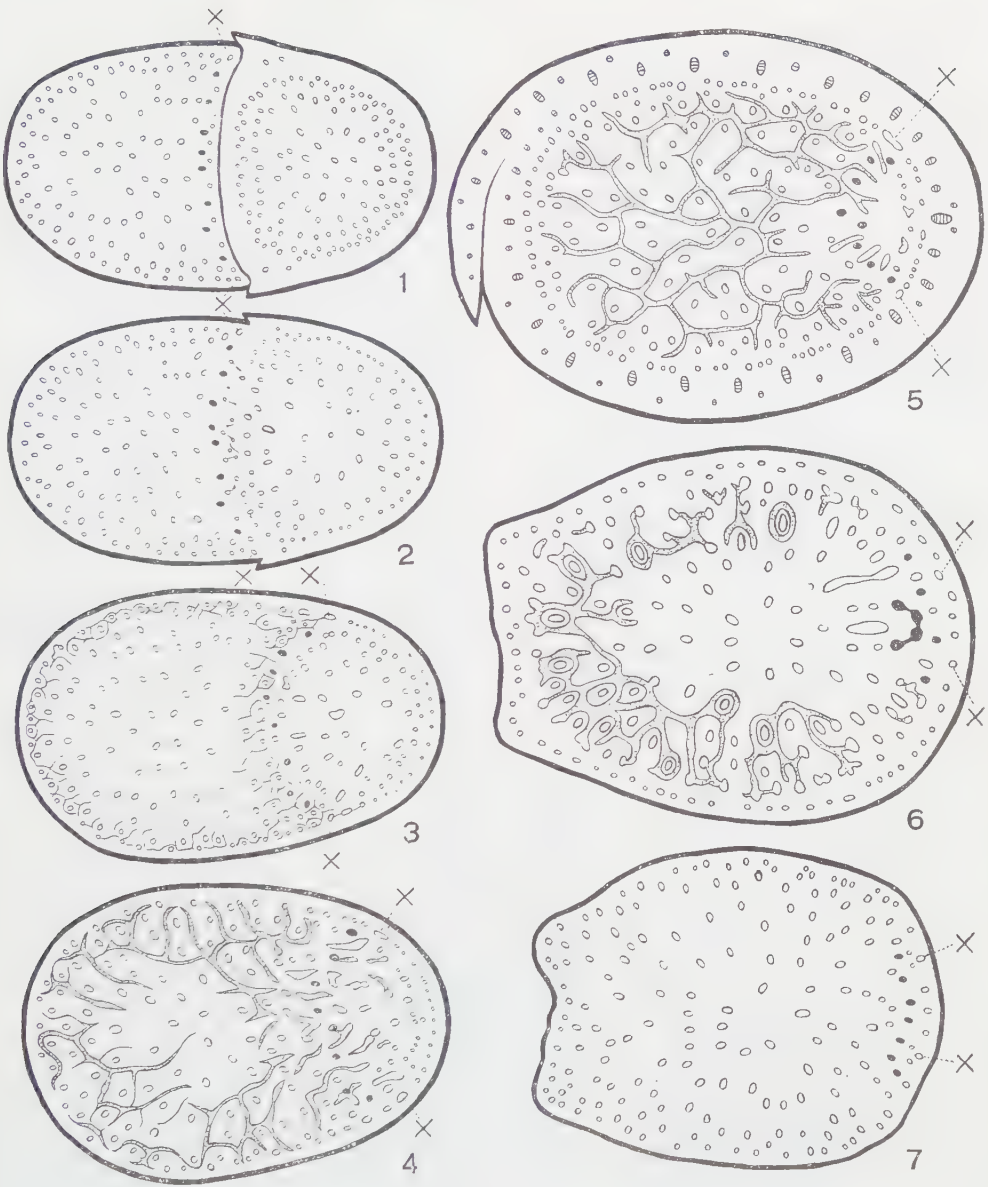


Fig. 1. Transections at various levels of the culm, beginning with the base of an axillary shoot (1), through the node and ending below the node (7). 1: left half of the figure, the mother axis (culm); and right half, the base of axillary shoot (shank). Compound bundles (inner system), situated at the side facing the axillary shoot, in 1 are shown in black. Trace bundles from the subtending leaf are shaded in 5, and the horizontal nodal plexus stippled. x: angle bundles.

側枝側に位置するもののみが黒く示してあり、又合成維管束よりも茎の表面近くに存在する最辺周部維管束の内、母軸の両稜角に位置するものには特に×印がつけてある (Fig. 2, 1 でも同様)。この維管束を記述の便宜上稜角維管束 (Angle

bundle) と呼ぶことにする。

母軸と側枝の維管束結合を知るために、節の上部から下部へ横断切片をたどるに、先づ最初に母軸と側枝との最辺周部維管束の内、相互に相對する方向のものが更に接近して結合し、そのまゝ消

滅するものもあるが (Fig. 1, 2), 結合したものの多くは主軸と側枝との接着線に副うて横走する。Fig. 2, 1 の n はこれを示すが, 図にはその走向は示してない。この横走小維管束の1部は葉節部に固有のいわゆる節網維管束と混合して行方を見失うが, それ以外のものは2群に別かれて結局稜角維管束に併合される。Fig. 1, 3~6 では節網維管束が不規則に横走しているが, これは側枝の有無に拘らず, 葉の着生する節に限って現われるものである (第4報)。従ってこれと母軸・側枝間の最辺周部維管束の結合に伴つて生ずる前記の横走小維管束とは本来別系統に属すると考えられるが, 実際には両者が立体的に或る程度相互に錯綜している。稜角維管束は下方に至るに従い, 側枝の背軸側最辺周部維管束を併合しつつ相互に接近して (Fig. 1, 4~6), 節の下部の節間では Fig. 1, 7 に見る如く両者の間には少数の最辺周部維管束を挟むに過ぎないことになる。

側枝内の髓内維管束 (葉跡及び合成維管束) は側枝節部で相互に合着して, その数を減少しつつ

母軸の側枝側に位置する合成維管束 (Fig. 1 で黒く示してあるもの) と結局併合されるが, これと同時に母軸の中央部から横走し來った節網維管束とも接触する。以上のような経過で枝跡条の大部分は独自の存在を抹殺されるので, 結局節の上部で側枝側に位置していた母軸の合成維管束は枝跡条を受け入れた節部以下の水準 (Fig. 1, 7) においても, やはり最辺周部維管束のすぐ内側に, 大凡1輪をなしてその位置を保っている。

要するに側枝中の維管束は母軸の節部において若干のものは節網維管束と接触するが, 本来節網維管束は第4報 (1942) で示したように, 多くは2次的分化のものであると云う理由から, 上記のような節網維管束との接触を一応考慮外において考えれば, 側枝の最辺周部維管束・合成維管束はそれぞれ母軸の最辺周部維管束・合成維管束と結合すると云える。

茎軸性側生器官である雄性小穂と母軸との維管束連絡の状況は第1報 (1939) 第2報 (1940 a) で示してあるが, それを模式図にしたものが Fig.

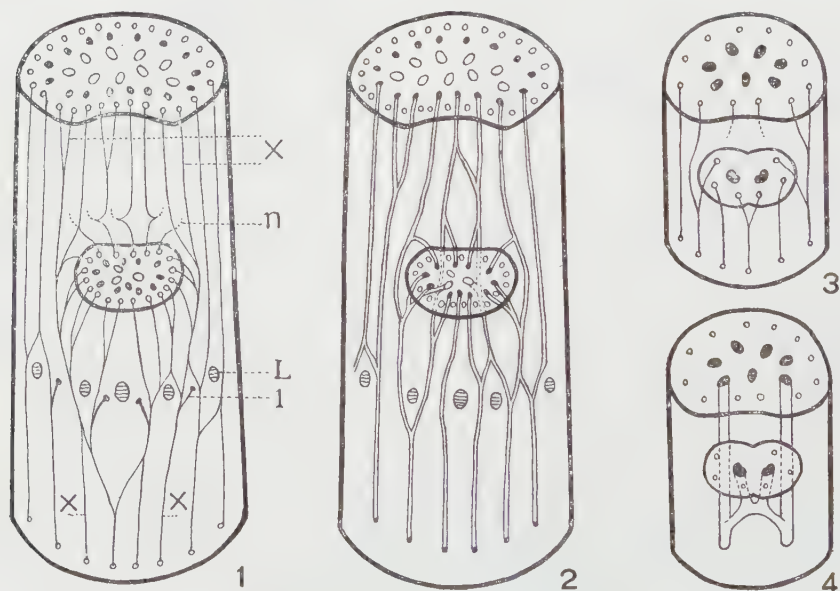


Fig. 2. Diagrams showing the vascular connections of the axillary shoot (1 and 2) and the fused base of paired spikelets (3 and 4) with their mother axis. Vascular connections of outer and inner systems are shown in separate figures. The base of the lateral organ is shown as a cut end, the compound bundles in black. Horizontal nodal bundles are not shown except at the part indicated by n . L and 1 ; large and small trace strands from the subtending leaf; the former follow inward courses and the position of entrance alone is shown here. X : angle bundles.

2, 3~4 である。これは 1 対の同位小穂の共通基部に見られる 2 個の中央維管束と数個の辺周部小維管束との雄花穂軸維管束に対する連結関係を示すために 2 図に画き分けてある。母軸内の内輪をなす大型維管束は第 1 報で茎軸維管束とみなし、第 4 報 (1942) 以後では合成維管束と呼んで来たものに相当し、外輪をなす小維管束は第 2 報以来最辺周部維管束と呼んだものに相当する。この小穂が母軸に着生する場合には維管束の数が少く、葉跡や節網維管束が見られないため、両器官の維管束連絡は簡単である。これを Fig. 2, 1~2 に示した葉腋の側枝の場合と比較する時、両者の間に簡複の差はあっても、型式に本質的差異のないことが明かである。

枝 跡 条 の 発 生

トウモロコシの腋芽の初期組織分化は Ledin (1954) が研究しているが、母軸・腋芽間の維管束連絡の過程については触れていないので、以下この点について述べる。

既に第 5 報 (1946) で詳記したように、茎の 1 断面において中心部に近い位置から辺周部の外向へ小維管束が順次出現し、大維管束としての成熟もその順序に従う。最辺周部維管束は前維管束としての出現も、その後の組織成熟も最後になる。これは腋芽内についても全く同様である。腋芽の始原体ではその中心部に最初少数個の前維管束が分化するが、これを下方へ追跡すれば次第に分化程度が低下し母軸の組織中に入ると間もなく* その存在を指摘できなくなる。側枝分化のこの時期には、母軸のその付近は基本分裂組織中に合成前維管束の一部のものが求頂的発生をなしている。更に後の時期では側芽よりの前記の合成前維管束の求基的分化は進行し、遂に母軸内の合成前維管束に連絡し、又一部は丁度その頃、前維管束の状態でその附近に出現する節網維管束とも接触して、枝跡条としての自立性を失う。側枝内では前述の髓内条におくれて、背軸側最辺周部維管束となるべき前維管束が現われ、これが髓内条と同様求基的分化をなし、一方丁度この節附近まで

求頂的に分化して来ている母軸の最辺周部の前維管束と連絡する。但し母軸の最辺周部維管束となるべき前維管束自身の分化は、厳密には必ずしも連続的の求頂的とは限らず、少くとも一部のものは節間最下部で、稍その分化が甚だ短時間ながら、第 5 報 (1946) で示したように遅滞する。従つて分化初期の母軸の最辺周部前維管束は節間の一部から出発し、求基的及び求頂的に分化する時期があることになる。これは次の所見から明らかである。

即ち側芽の向軸側最辺周部維管束と母軸の側芽側最辺周部維管束とを、節の上部から下方へ横断切片で追跡すると、母軸と側芽との合着点附近の基本分裂組織中で、両者の最辺周部維管束が共に前維管束としての存在を指摘出来なくなる。更に少し側芽が発達した階段では、両者が相結合し、その後 Fig. 2, 1, n に示したように若干横走し始める。これはその附近に分化し始めた節網維管束と併合するものもあるが、多くは 2 群に別れて稜角維管束 (Fig. 2, x) に併合される。

側芽の発育の初期には前述の如く母軸・側枝間の直接の維管束連絡は両者が相互に相対面する方向のそれぞれ最辺周部維管束相互間或は合成維管束相互間に見られるのみで、枝跡条が母軸髓中を横ざり、反対側の母軸維管束に合着する確実な所見は得られない。然し側枝が強大に発育した場合には、側枝の向軸側に位置する最辺周部維管束、又は小維管束を下方へ追跡すると、それが出線を描いて母軸中央を横切つて横走し、反対側に移つて下降することを、縦断切片で比較的明白に指摘できる場合がある。これは側芽の発育初期では、母軸と側枝との維管束連絡が先ず相互に相対面する 1 側面に起き、側枝の発達に伴い、母軸・側枝間の連絡緊密化の必要に即応して、節網維管束の一部が恰も枝跡条の直接の連絡かの如く、2 次的に強化された結果と思われる。

主幹の下部の葉節では、腋芽が分化早々その発育を停止することが多いが、この場合には停止した時期の早晩の差により、腋芽中に数個の髓内条

* 第 5 報では側脈に由来する葉跡条の前維管束は求基的に分化し、母軸中の前維管束とは節部で不連続であり、後にこれと連絡することを述べ、この不連続は節部における組織分化の遅滞することに帰した。この場合組織学的にも形態学的にも未だ節間の分化のない部位における所見であるから、理論的には節部と呼ぶよりは Sharman (1942) の如く “disc of leaf insertion” の 1 部とでも表現した方が妥当と思われる。

のみが存在し、これが枝跡糸として母軸中に入っても、母軸の維管束と全く連絡をなさず、不連続のまゝに終ったり、或はこれが母軸維管束と或る程度の連絡をなしていても、それより遅れて分化する最辺周部維管束だけは、母軸のそれと不連続のまゝ血管で終っている。これらの場合、枝跡は維管束として完成せず、前維管束の状態でそれ自身の分化を停止し、周囲の柔組織が成熟するため、これに包摂されて殆ど吸収されたかの貌を呈し、辛じてその名残を認め得ることが多い。

要するに枝跡の前維管束は基本的に分化し、比較的小さく meristematic である節間最下部附近で、同じ頃前維管束として分化してきた母軸の合成維管束・最辺周部維管束及び同じく前維管束として分化し初めた節網維管束とも連絡する。従って分化時期の早い母軸の中央附近に存在する維管束に対しては、枝跡が直接連絡の機会がないわけである。

不定根の発生

不定根は節間の下部即ち伸長帯に接近した部位に生ずるが、その形成発端の時期は極めて早く、母茎の該部において、最辺周部維管束が前維管束の状態として、基本分裂組織中に分化を開始すると殆んど同時である。第 5 報 (1946) で述べた通り、横断面の所見では、茎の維管束が瓶の中央に位置するものから大体に順次遠心的に分化が進行し、最辺周部維管束が最後に分化する。従ってこれが出現する筈の皮層に内接する環状の部分は、比較的永く基本分裂組織の状態に留まつている。而してこの環状帯の内に点状に最辺周部維管束が分化するわけであるが、同時にこの環状帯を構成する細胞群の内、外部に位置すると共に、切線方向へ連なる 1 連の細胞群が、放射方向へ分裂して、不定根の発端となる。茎の皮層内に内皮が分化せず、従って皮層と中心柱との境界が不明であるから、この不定根の発端となる細胞群の組織学的帰属を確定することができず、唯最辺周部維管束帯に外接していることを云い得るに過ぎない。

同一水準から多数の不定根が輪生するのが普通であるから、不定根発端分裂組織は茎の横断面では中心柱の全周に亘り、断続した環状帯をなすことが多い。この環状帯の諸所が後に厚さを増して山型に突出し、その頂端が根端分裂組織となり、常例の如く皮層を通り表皮を破って頂端成長を行

い、外部へ不定根として姿を現わす。環状帯の 1 部が突出し出す前後の頃、この環状分裂組織帯と分化したばかりの茎の合成維管束との間に横走する前維管束が出現し、これが両者を結合させると共に、その横走前維管束は合成維管束の位置よりも更に若干髓の中心方向へも伸長するが、余り髓の深部までには発達せず、又合成維管束以外の髓内条とも専断として結合しない。環状分裂組織の大部分は、後に維管束及びこれを取り巻く機械組織となるが、部分分裂組織が前述の如く、最辺周部前維管束と相伴って分化したものであるから、前者が根糸条となつた暁には、最辺周部維管束と密接な結合のあることは当然である。

成長した不定根と茎との維管束連絡

Fig. 3 (は充分成長した 1 個の不定根の着生部位のすぐ上部 (1) から、その根が大凡縦断される水準 (4) までの間の横断面であって、茎の表皮附近の 1 部と 1 個の不定根の基部とが示されている。図中細点密布の部分は維管束の木部・篩部を示し、その周囲を環状に或は半月形に取囲む黒色部分は、茎の合成維管束に伴う厚膜維管束鞘であるが、同様の維管束鞘でも、それ以外の維管束に伴うものは黒くしてない。紙面に平行に走る維管束にも維管束鞘を伴うから、これは全く図示してない。図の明らかなように、根の維管束は茎の最辺周部維管束及び合成維管束と直接又は横走維管束を経由して連絡するが、それ以外の維管束とは原則として連絡しない。この不定根の着生に伴う横走維管束は葉節に見られる節網維管束とは位置的に分離していて、相互に連絡がないので、両者は本来別系統のものと考えられる。然し節間成長が著しくない場合には、両系統が位置的に上下に接触していて、相互に連絡していると想像されるが、この点確認してない。

不定根の維管束が原則として茎の最辺周部維管束及び合成維管束以外の垂直維管束と直接連結しないことは、茎の髓の中心に近い位置の維管束の分化が、辺周部のそれよりも時間的に早いといふ第 5 報 (1946) の所見から当然の結果と考えられる。

尚、根跡は母軸の維管束から由来しても、母軸維管束条の走行その他に何等影響を与えない点で、枝跡や葉跡と著しくその趣を異にする。

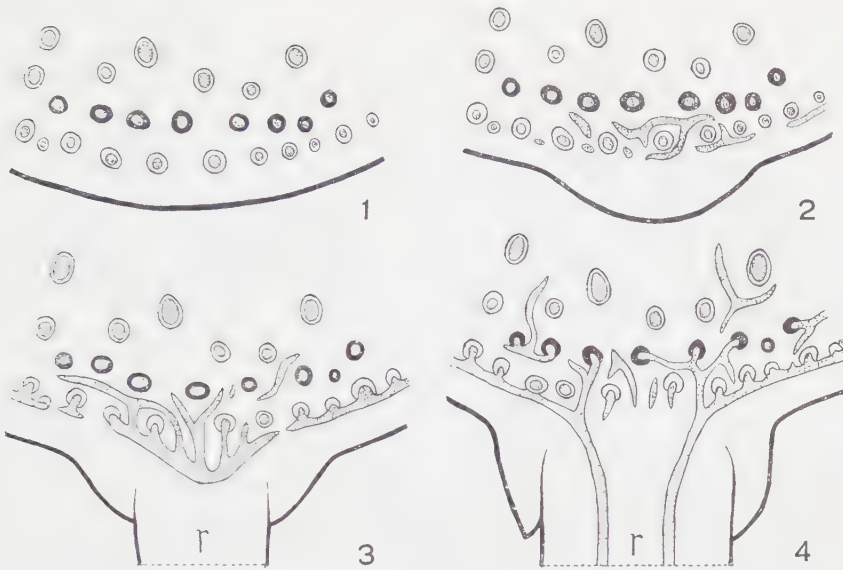


Fig. 3. Transections of a part of the culm at various levels from above to below, showing the vascular connection of the adventitious root with the stem. The base of adventitious root (*r*) is longitudinally cut in the last figure (4). Vascular bundles or procambial strands stippled. The fibrous sheaths of compound bundles (inner system) shown in black, those of other vertical bundles in white, those of horizontal bundles not figured.

Summary

The procambial strands, initiated at the base of the axillary bud, elongate basipetally and are connected with those of the mother axis. A few branch traces follow the horizontal courses at the node, or become blended with the nodal plexus, and thus their further accurate behaviours are difficult to trace. The nodal plexus is of the secondary origin in the histogenesis, and if those branch traces are left out of consideration, most of the trace strands from the axillary bud are connected, as a rule, with the vertical bundles of the mother axis at the node; i.e. the outermost peripheral (outer) and compound (inner) bundles of the axillary bud are connected respectively with outer and inner axial bundles situated at the side facing the axillary bud. A few branch traces pass through the node downwards without being connected with any bundle of the mother axis. In the case of the developed axillary shoot, some branch traces seem to pass across the centre of the mother axis and join directly with the axial peripheral bundles, situated at the side opposite to the axillary shoot. As such a behaviour of branch traces is not confirmed in the early stage of the bud, it is interpreted as a secondary modification caused by the predominant development of one of the nodal horizontal strands which are initiated between some branch traces and vertical axial bundles. The pattern of the vascular connection of the axillary shoot with its mother axis will be discussed in the following paper of this series.

Before the intercalary elongation of the shoot takes place, an arc of meristematic tissue becomes initiated closely attaching to the outermost peripheral procambial strands of the stem, and later it develops into a dome-shape. From the summit of the dome the adventitious root apex is initiated, and then some horizontal procambial strands are differentiated between several points of the dome base and its adjacent compound procambial strands of the stem; thus the vascular connection of the stem with adventitious root is established. The horizontal strands above mentioned usually elongate more deeply towards the centre of the stem, but they are not connected there with any of vertical axial bundles.

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本 会 記 事

昭和 32 年度会計決算報告（昭和 32 年 1 月から昭和 32 年 12 月まで）

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朝 日 科 学 奨 励 金 に つ い て

朝日新聞社から朝日科学奨励金候補研究の募集について推薦の依頼がありましたので、会員各位の中で
適当と思われる研究がありましたら、所属支部の評議員の推薦状を添えて 3 月 15 日までに学会本部宛に
御連絡下さい。

● 訃告 ● 松浦茂寿氏は昭和 32 年 9 月 29 日死去されました。
● 訃告 ● 徳田省三氏は昭和 32 年 12 月 16 日死去されました。
ここに報告し謹んで哀悼の意を表します。
日本植物学会

本年夏より本会九五支等は、前記のように行いました。
福岡県博多区南門外 九五支会（福岡市立第一小童謡研究會）

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中山弘美	東京都文京区第六天町 8 第六天 住宅 145 号	吉岡俊三	福岡市香住ヶ丘 1 福岡女子大 生物

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浅 野 一 男 長野県下伊那郡 山吹小学校
川 豊 康 新宿区早稲田鶴巻町22 大泉方
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上 星 貢 札幌市琴似町西 8 軒56 高橋方
久 保 淳 福岡市前原町篠原
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立水族館
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工 藤 照 夫 弘前市坂本町 5 弘前学院短大
生物
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小学校
豊 田 清 修 藤沢市鶴沼 2957
新 関 宏 夫 平塚市 農技研
田 村 博 美 鹿児島市加治屋町 107 鶴丸高
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高校
内 田 恵 子 熊本市大江町渡鹿 768
藤 茂 宏 岡山大理生
秋 山 優 松江市西川津町 島根大文理生
物
朝 倉 勇 広島県佐伯郡五日市町 藤垂園
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寺 内 康 三 茨城県水戸市第一中学校
佐 宗 守 神奈川県足柄上郡中井村立中井
中学校
荒 野 久 雄 埼玉県北足立郡足立町 1823
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田 辺 至 北海道留萌市千鳥町 留萌高校
内
秋 田 茂 雄 札幌市北 28 条東 3 丁目
梅 楚 国 夫 福岡市博多区 福岡高校
梶 良 美 神戸市兵庫区有野町上向山
宮 田 源 長野市中千田 183 堀内方
村 松 享 松本市西町 482
川 崎 次 男 相模原市上鶴岡 5648
吉 井 義 次 岐阜市外那加町岐阜大本部
増 田 芳 雄 大阪市立大・理工・生物
加 藤 勇 夫 広島大・教養・生物
村 山 徹 郎 愛媛大・文理・生物
津 山 尙 文京区小日向台町 1-44
上 屋 茂 高田市稲田北陸農業試験場
奥 富 清 福岡県田川郡伊田福岡学芸大田
川分校・生物
尾 形 英 三 下関市吉見町農林省水産講習所

Physiological Studies on a Thermophilic Blue Green Alga, *Cyanidium caldarium* GEITLER*

by Ikujiro FUKUDA**

福田育二郎**: 温泉性藍藻, イデユコゴメの生理学的研究*

Received November 4, 1957

Introduction

The unicellular blue green alga, *Cyanidium caldarium* GEITLER, represents one of the thermophilic forms of Cyanophyceae of wide distribution, often showing luxuriant growth in various hot springs in this country (Negoro 1944). The organism is remarkable also in its acidophilic nature, tolerating even an acidity as extreme as pH 2.0 in its natural habitat. Because of these natures, it can be isolated relatively easily from other microorganisms. Although it grows rather slowly under experimental conditions, its nature of growing uniformly in liquid media makes it suitable as the material of physiological studies.

In the present study, the effect of some environmental factors upon the growth and physiological activities of this organism were investigated.

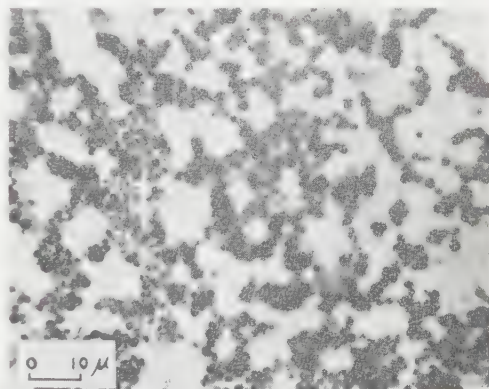


Fig. 1. *Cyanidium caldarium* GEITLER.
Two week old culture: Stained with
carbol-fuchsin.

Materials and Methods

Isolation of the organism The strain of *Cyanidium caldarium* GEITLER used in the present study was obtained from Yumoto-Spa, Nikko, Province of Shimotsuke and isolated free from bacterial contaminations by the following procedures.

Silica-gel plates Silica-gel was prepared after Fuhrmann's method with a modification of using sulfuric acid instead of hydrochloric acid. Solid medium for the isolation of the organism was made by adding sulfuric acid and other basal inorganic ingredients (see later) to the silica-gel thus prepared.

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** Botanical Institute, Faculty of Science, University of Tokyo, Tokyo, Japan. 東京大学理学部植物学教室

Isolation After repeated transfers on the surface of silica-gel plates, the subcultures were still found to include contaminations of certain aerobic bacteria. With the purpose of getting rid of these contaminants, successive subculturing under anaerobic condition (N_2 : 80, CO_2 : 20) and another subsequent culture through a liquid medium containing

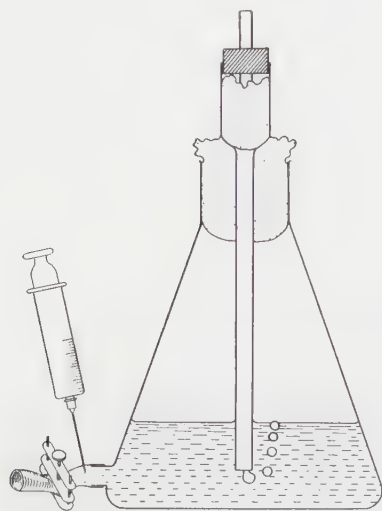


Fig. 2. Culture device.

air containing 5% CO_2 . The intensity of illumination was about 10,000 Lux; temperature of water bath was kept at 45°.

Composition of culture medium

$Ca(NO_3)_2$	0.5 g.
KH_2PO_4	0.2 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.
$FeCl_2$	trace
H_2SO_4 (1N)	10 ml
Distilled water.....up to	1,000 ml
(pH: 2.4)	

Growth was measured with aliquots of the culture sampled at intervals by means of a syringe as illustrated in Figure 2. Electro-photometric readings were converted into figures in dry weight (mg./ml), packed volume (ml/l), or algal cell number (N/ml), with the use of respective standard curves obtained through preliminary experiments.

Metabolic activities were determined with algal cells harvested and washed three times with hot, distilled water (45°) on a centrifuge, and resuspended in a citrate-phosphate buffer (pH 3.0, M/10). Usually 14 day old culture of the alga was used when not otherwise stated. The rate of gas exchange was measured with a Warburg manometer usually at 45°, with 10 to 20 ml of suspension containing about 50 or 100 mg. dry weight of algal cells. The use of the bicarbonate buffer ordinarily in use in the measurement of photosynthetic activity was impracticable because of high temperature and extreme acidity of the medium used in the present study. We worked therefore

a sublethal dosis of streptomycin (0.1 to 0.5 mg./ml) were carried out. The subsequent inoculation on a silica-gel plate gave rise to a culture of the alga successfully freed from any contamination. Purity of the culture from the contaminants was tested by microscopic examination, and by inoculation on an ordinary nutrient agar containing peptone-broth or yeast extract. Throughout these procedures, cultures were continuously illuminated with the light from a incandescent lamp (intensity of illumination: ca. 10,000 Lux).

Culture in liquid medium The organism was grown in a cotton plugged flask (Fig. 2) on an inorganic medium of the following composition, under continuous agitation of the culture by bubbling with

mostly with the *indirect method* of Warburg, taking the readings with 10 ml of the suspension in one cup, and 20 ml of it in another cup of the manometer. Sometimes the usual *approximation method* was also adopted, in which the difference between the gas exchanges observed in the dark and in the light was taken as the measure for photosynthetic activity.

Results and Discussion

Effect of the hydrogen ion concentration on growth rate The growth of the organism was found to be remarkably dependent on high acidity of the medium. One set of the representative results of the growth experiments is shown in Figure 3. Maximal growth was obtained with an initial pH of the medium as low as 2.0. With an initial pH of 4.0 the growth was much slower. At pH 5.0 there was a marked inhibition of growth. With this and with still lower level of hydrogen ion concentration, there appeared indications of injurious effect, the inoculated algal cells being decolorized after a few days.

From the results of the experiments in which sulfuric acid of the growth medium (see above) was replaced by hydrochloric acid, it was revealed that the high acidity, not necessarily the presence of sulfuric acid, was the requirement for the favorable growth of the organism.*

Effects of environmental conditions on photosynthetic and respiratory activities The rates of photosynthesis and respiration are shown in Figure 4 as functions of hydrogen ion concentration of the reaction medium. The experiment was carried out with two week-old culture of the alga, grown in a medium with an initial pH of 3.0. The washed cells were suspended in a series of citrate-phosphate buffers of pH 2, 3, 4, and 5 and the activities were measured as described above. The maximum velocity of photosynthesis at an acidity as extreme as pH 3.0 was noticed. The respiratory activity as measured by the rate of oxygen-uptake in the presence of citric acid as one of the buffering substances, was also found to be maximal around pH 3. All the following

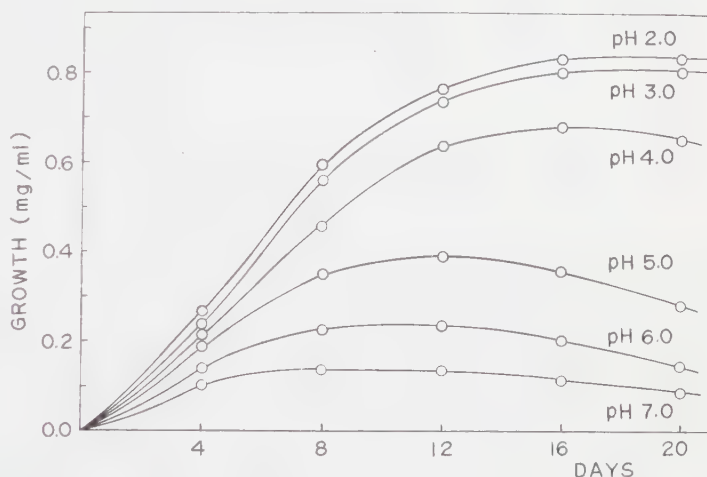


Fig. 3. Time course of growth of *Cyanidium caldarium* GEITLER with varied initial pH of the medium. (Temperature of incubation: 45°).

* It was noticed that during the culture the pH of the culture medium was shifted towards neutrality. The shift, however, did rarely amount to 0.8 unit of pH.

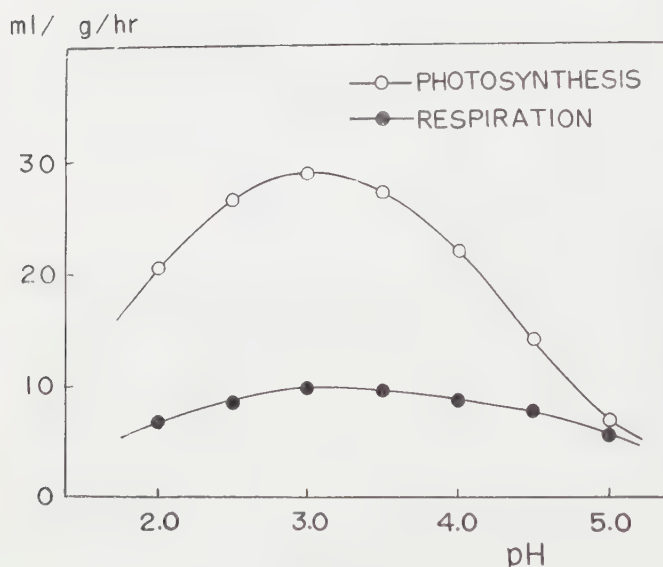


Fig. 4. Rates of photosynthesis and respiration of *Cyanidium caldarium* GEITLER, as influenced by pH of the reaction medium. (Temperature: 45°, Intensity of illumination: 10,000 Lux, Partial pressure of CO₂ in air: 5%, in photosynthetic experiments).

From the results shown in the figure, it will be noticed that the photosynthetic activity of the organism was subject to a sudden and significant drop at a point when the stationary stage of the growth curve was reached. Actually, this stage of culture was reached usually after about two weeks of growth, when the cells were harvested and used for the studies. The respiratory activity also changed, but in the reverse sense:

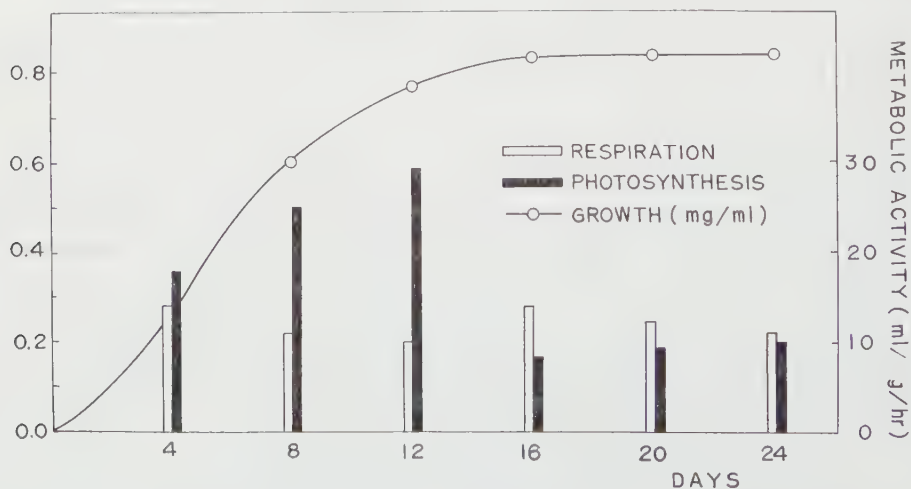


Fig. 5. Metabolic activities of *Cyanidium caldarium* GEITLER, at different stages of culture (Both at pH 3.0, 45°, Conditions of measurements: the same as given in Fig. 4).

measurements of the metabolic activities, therefore, were run at pH 3.0.

Changes in metabolic activities during the course of culture The changes in respiratory and photosynthetic activities of the organism during the course of culture are shown in Figure 5. Both activities are presented as the respective gas-exchange per unit weight of algal cell (ml/g/hr.), and compared with the growth curve of the culture, from which the samples were taken at 4 days' intervals to be investigated as described above.

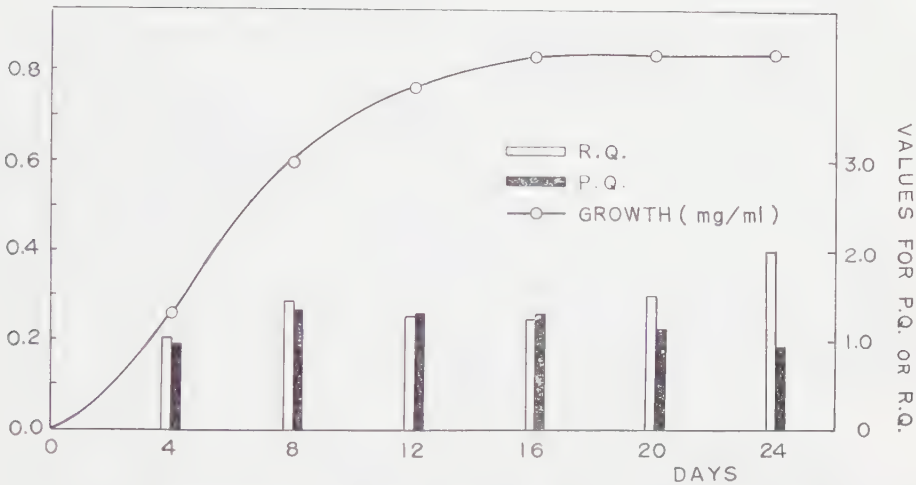


Fig. 6. Photosynthetic and respiratory quotients of *Cyanidium caldarium* GEITLER, at various stages of culture (replotted from the data in Fig. 5).

the rate (per unit algal mass) was larger in earlier stages of growth, and reached a minimum at the beginning of the stationary stage of growth, to increase with the further age of the culture. There were also certain changes in respect to the values of photosynthetic and respiratory quotients with the age of culture (Figure 6). At the stage of culture at which most of the test materials were harvested, (i.e. at 12 to 14 days of culture), the cells were obtained with levels of respiratory and photosynthetic quotients both around 1.3.

Effect of carbon dioxide concentration and light intensity on the rate of photosynthesis The rate of photosynthesis was measured at 45° (pH 3.0) under varied CO₂ partial pressure and under varied intensities of light. The light intensity was measured immediately at the bottom of manometer flasks with a Matsuda Lux Meter. (Figures 7 and 8). Characteristic figures in respect to CO₂-partial pressure were found to be as follows:

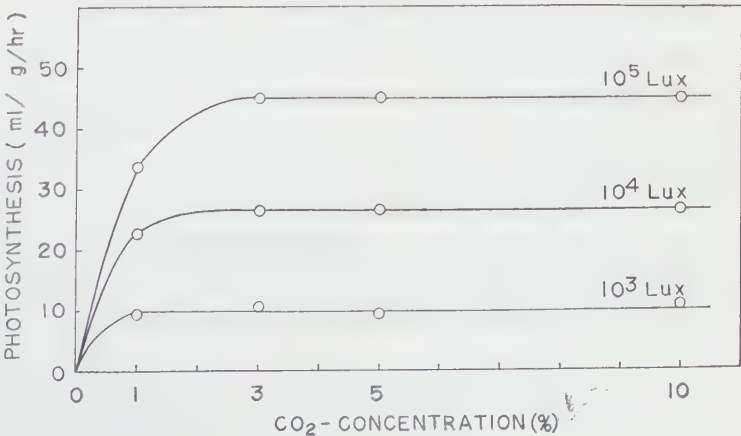


Fig. 7. Rate of photosynthesis of *Cyanidium caldarium* GEITLER, as influenced by carbon dioxide concentration (pH 3.0, 45°).

Half-saturating CO ₂ -concentration	Saturating CO ₂ -concentration	Light Intensity
1.2×10^{-4} mol/l (0.6% in air)	ca. 6×10^{-4} mol/l (ca. 3% in air)	10 ⁵ Lux
8×10^{-5} mol/l (0.4% in air)	ca. 4×10^{-4} mol/l (ca. 2% in air)	10 ⁴ Lux
	ca. less than 2×10^{-4} mol/l (ca. 1% in air)	10 ³ Lux

(pH 3.0, temperature 45°)

These figures are remarkably higher than those reported in the case of the green alga, *Chlorella vulgaris* (Warburg, 1919, 1920).

Half-saturating CO ₂ -concentration	Saturating CO ₂ -concentration	Light Intensity
2.6×10^{-6} mol/l	9.1×10^{-5} mol/l	10 ⁴ Lux

(bicarbonate buffer, temperature 25°)

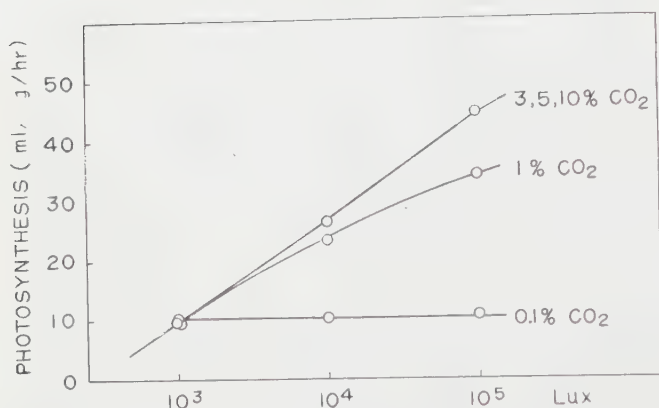


Fig. 8. Rate of photosynthesis of *Cyanidium caldarium* GEITLER, as influenced by light intensity (pH 3.0, 45°).

reached at a light intensity of about 10⁴ Lux. With still lower concentrations of carbon dioxide, 10³ Lux of light intensity was found to be sufficient to light-saturate the photosynthetic system involved. Generally speaking, the requirement for light energy with the blue green alga under investigation seems to be much higher than that with the green alga, *Chlorella*. In the latter case, the photosynthetic activity of *Chlorella vulgaris* in the presence of saturating amount of carbon dioxide has been reported to be saturated at an light intensity as low as 10,000–20,000 Lux (Warburg, 1919, 1920). In the case of *Chlorella ellipsoidea*, it has been also represented to be saturated at 15,000 Lux (pH 10, temperature 25°), and half-saturating concentration at 3,000 Lux (Tamiya and Chiba, 1949).

Effect of temperature on photosynthesis The rates of photosynthesis and respiratory oxygen-uptake are presented in Figure 9 as functions of reaction temperature. The

The results of the experiments shown in Figure 7 are replotted in Figure 8 in respect to relationship between the rate of photosynthesis and light intensity. With a concentration of carbon dioxide as high as 3% CO₂ or more in air, the rate of photosynthesis increased linearly with logarithms of light intensity. With 1% CO₂ in air, saturation was

extraordinarily high temperature of maximum activity with this thermophilic organism is to be noticed. The highest values for the rates of either activities were obtained at 55°. In Figure 10 the logarithms of the photosynthetic rate ($\log v$ in the figure) are plotted against the reciprocal of reaction temperature ($1/T$). These experiments were performed with minute amounts of carbon dioxide and under saturating intensity of illumination. The Arrhenius plot presented in the figure shows us, therefore, the apparent heat of activation ΔH^* pertaining to the "dark reaction" of photosynthesis under investigation. The results of the computation were as follows,

$$\begin{aligned} \Delta H^* &= 17 \text{ Kcal} \\ & \quad (55^\circ\text{--}40^\circ, \text{pH } 3.0) \\ \Delta H^* &= 7 \text{ Kcal} \\ & \quad (40^\circ\text{--}30^\circ, \text{pH } 3.0) \end{aligned}$$

In the range of temperatures below 25°, there is a more abrupt change of the reaction rate with the temperature, corresponding to a value for the apparent activation energy of about 70 Kcal. Certain secondary change in the photosynthetic mechanism must be taking place in this range of temperature, under which no measurable growth is ever possible with the organism under investigation.

The rate of respiratory oxygen-uptake was less markedly influenced by the temperature change (Figure 9). Nor any abrupt decrease in the reaction rate observed at temperatures below 25°.

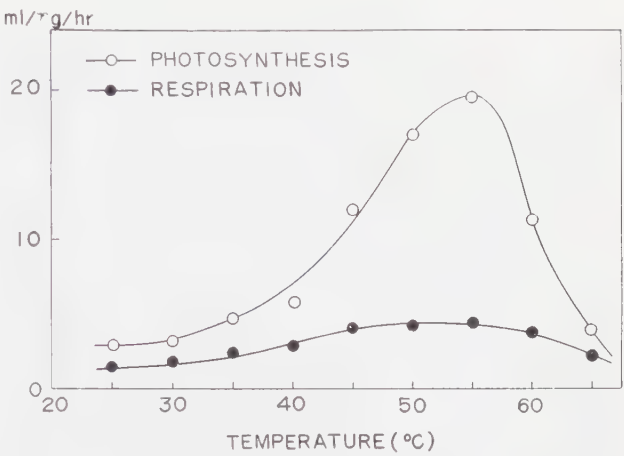


Fig. 9. Rate of photosynthesis and respiratory oxygen-uptake of *Cyanidium caldarium* GEITLER, as influenced by reaction temperature (pH 3.0, Photosynthesis: under saturating light intensity; ca. 0.1% CO₂ in air).

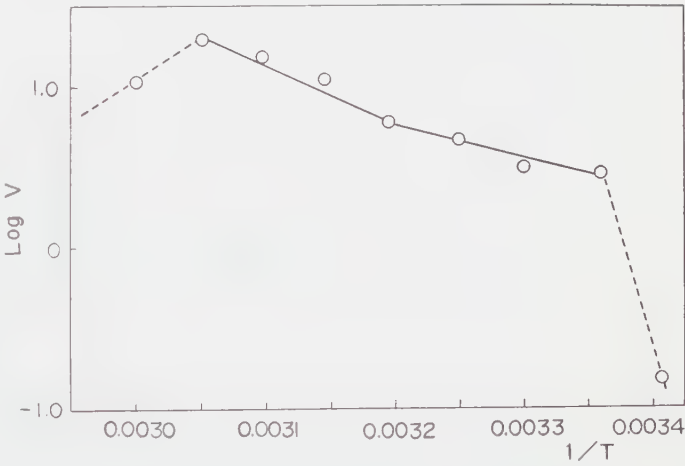


Fig. 10. Rate of photosynthesis of *Cyanidium caldarium* GEITLER, as influenced by reaction temperature (pH 3.0); Arrhenius plot. $\log v$: logarithm of photosynthetic rate (ml/g./hr.).

Summary

A strain of blue green alga, *Cyanidium caldarium* GEITLER, was isolated from the sample material collected from a hot spring in Yumoto, Nikko, Japan. Consistent sub-cultures were obtained and the growth responses of the organism towards various environmental conditions were investigated.

The alga was found to be markedly thermophilic and extremely acidophilic in nature, most favorable growth taking place at 45°~50° and pH 3.0.

The changes in the metabolic activities of the organism during the course of culture were followed.

The effect of hydrogen ion concentration, temperature, light intensity and carbon dioxide concentration on the rate of photosynthesis was investigated. The optimum hydrogen ion concentration was pH 3.0. The optimum temperature lied at 55°. The apparent heat of activation (in respect to the dark reaction of photosynthesis) amounted to 17 Kcal for the temperature range 55°~40°, and 7 Kcal for 40°~30° (pH 3.0). The saturating intensity of the light was found to be about 10⁵ Lux or higher with sufficient amount of carbon dioxide in the medium. Under sufficiently strong illumination, the half-saturation of photosynthesis was reached at a concentration of carbon dioxide of ca. 1.2×10^{-4} mol/l (or about 0.6% CO₂ in air: pH 3.0, 45°). These findings were compared with the data reported for the green alga, *Chlorella*.

The author wishes to express his cordinal thanks to Prof. H. Tamiya, Prof. A. Takamiya, and Dr. S. Morita of University of Tokyo and to Prof. H. Huzisige now in Okayama University for their kind guidance and encouragement. Thanks are also due to Dr. H. Fukushima of Yokohama Municipal University for the identification of the algal organism.

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On *Streptomyces spiroverticillatus* nov. sp.*

by Ryuji SHINOBU**

信夫隆治**：新種 *Streptomyces spiroverticillatus* ついて*

Received November 12, 1957

I. Morphology

Many kinds of media, such as sucrose ammonium agar, glucose asparagine agar, Camalate agar, potato peptone glycerine agar etc. were used for the microscopical observations. Though on sucrose ammonium agar, the growth of these strains were moderate and thin, it is not too much to say that this medium is one of the most suitable one for the microscopical observation, because of the fact that thin white aerial mycelium forms many whirls and they can be easily seen on the skirts of the colony through the direct observation method.

Glucose asparagine agar was good for the growth of the colony and it was also suitable for the micromorphology. Therefore, these two media were mainly used for the morphological studies.

The experiment was carried on the colony incubated for about 7~10 days in 28°~30°. The author adopted chiefly the direct observation method in which the colony plated on the petri dish was made to grow fully. Sometimes, other methods were parallelly used.

1. Aerial mycelium: generally, long and cottony on most media, sometimes fairly cottony, white, about 0.8μ in width.
2. Many spirals on sucrose ammonium agar, glucose asparagine agar and other media.
Spiral: generally, curled tip with 1~2 turns, seldom 3 turns, diameter of spiral about $5\sim 8\mu$, sometimes snail-like and hook-like curl. Occasionally, loose or closed spirals with 2~3 turns; sinistrorse.
3. Whirl formation: usually occurred near the base of the aerial mycelium, but generally, not so remarkable as the other whirl forming species; occasionally, very few tufts on the skirt of the colony.

Whirl: Nittela-type whirl; generally, primary whirl only, seldom secondary one.

Radial branch: about 2~4 short branches.

4. Conidia: spheroid, somewhat ellipsoid; about 0.8μ in length.

Conidiophore: generally very long.

Conidial formation begins in 3~5 days; conidia are formed at the top of the spiral

* The outline of this species was already reported at the 22th General Meeting of Botanical Society of Japan (1957).

** Hirano Branch, The Osaka University of The Liberal Arts and Education, Osaka, Japan.
大阪学芸大学平野分校

Photo. 1.

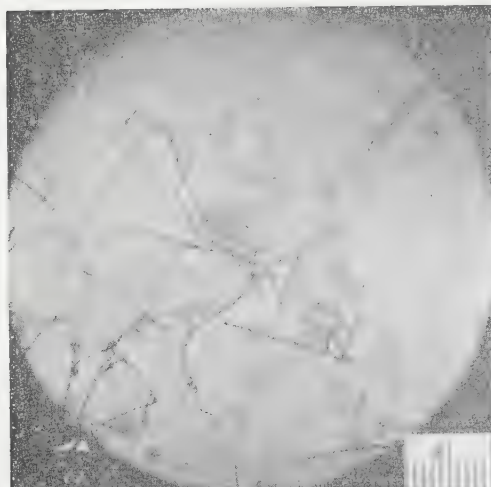


Photo. 2.

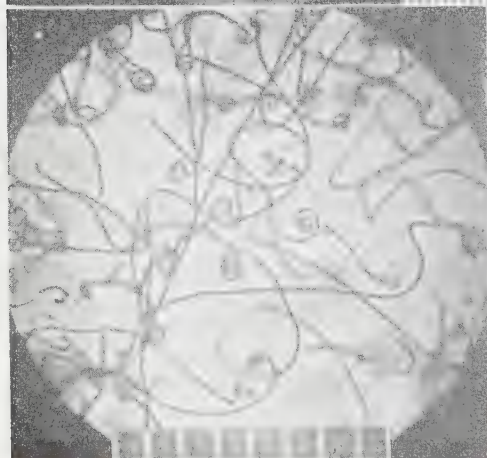


Photo. 3.

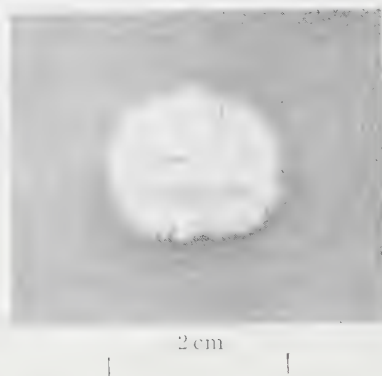


Photo. 4.

Photo. 1. Whirl and spiral, medium: sucrose ammonium agar, 10 days.

Photo. 2. Tuft and whirl, medium: sucrose ammonium agar, 10 days.

Photo. 3. Spiral; curled tip, snail-like, and hook-like, medium: sucrose ammonium agar, 10 days.

Photo. 4. Colony, medium: potato peptone glycerine agar, 15 days.

and the whirl's branch to the direction of its root.

5. Substrate mycelium: monopodial, no fragmentation; about 0.6μ in width.

II. Cultural and physiological characters

(1) Potato peptone glycerine agar

G: good; thick

A: good; cottony; white

S: at first, yellow orange~Golden Yellow; later, Buff~Golden Yellow

P: usually, none; sometimes pale brown

(2) Bouillon agar

G: good~moderate; somewhat thin

A: none

S: dull yellow orange~Corn
~Golden Yellow

P: none

(3) Nutrient agar

G: excellent, thick

A: none

S: Ambergrow~Golden Yellow~Buff

P: uncertain, probably pale brown

(4) Glucose peptone agar (Waksman's B)

G: good~excellent

A: good; somewhat cottony, white~brownish white

S: dark yellow orange~Ambergrow

P: pale dark yellow orange~pale brown

(5) Glucose broth

G: moderate~good; sometimes submerged

A: good; ring, cottony, white; when colonies submerged, no aerial mycelium

S: pale dull yellow~colorless

P: none

(6) Starch agar (Waksman's A)

G: good

A: good; cottony, white~brownish white~pinkish white

S: pale dull orange~light brown~Golden Yellow

P: none

(7) Glucose asparagine agar (Krainsky's)

G: good~excellent

A: good; cottony, white~brownish white

S: pale yellow orange

P: none

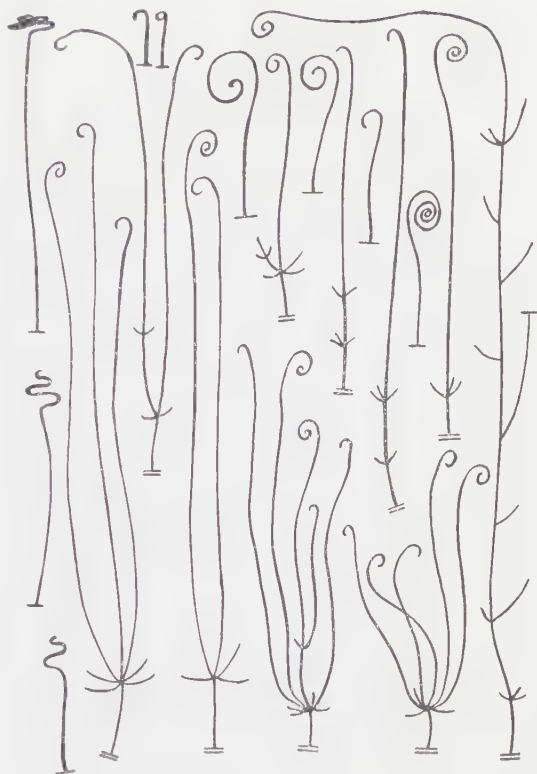


Fig. 1. Sketch of aerial mycelium, medium: sucrose ammonium agar, 10 days.

Notes { Ggrowth of colony
Aformation of aerial mycelium
Scolor of substrate mycelium
Psoluble pigment

(8) Ca-malate agar (Glycerine ammonium agar)

- G : excellent ; thick
A : abundant ; cottony, white
S : pale yellow orange~pale dull yellow orange~light brown
P : none

(9) Sucrose ammonium agar (Czapek's modified)

(2 grams of NH_4Cl as a substitute for NaNO_3)

- G : moderate~poor ; many little colonies
A : moderate~poor ; cottony, white
S : colorless~pale yellow orange
P : none

(10) Synthetic agar (Czapek's)

- G : moderate~poor ; thin
A : moderate~poor ; thin, somewhat cottony, white
S : colorless~pale brown~pale dull yellow orange
P : none

(11) Plain agar

- G : poor ; very thin
A : poor, thin, somewhat cottony, white~brownish white
S : colorless~pale brown
P : none

(12) Potato plug

- G : excellent ; thick, partially wrinkled
A : abundant ; cottony, white~brownish white
S : pale brown~brown~pale yellowish brown
P : brown

(13) Carrot plug

- G : good
A : good ; white
S : pale dull yellow orange~pale brown~brown
P : uncertain ; probably pale brown

(14) Egg medium

- G : moderate~poor ; partially ; wrinkled
A : scant ; white
S : colorless~pale brown
P : none

(15) Skimmed milk

- G : good ; sometimes submerged
A : poor ; light brownish gray ; when submerged, none
S : at first yellow ; later dull yellow orange
P : pale dull yellow orange

No coagulation; liquefaction rapidly

(16) Gelatin stab

G : poor; colony submerged

A : none

Liquefaction: strong

(17) Blood agar

Haemolysis: positive, weak

(18) Tyrosine agar

G : good

A : good; cottony; white~brownish white

S : at first dull yellow orange; later Buff

P : pale brown; sometimes none

Tyrosinase reaction: somewhat unstable, generally positive, weak

(19) Konjakmannan medium

No liquefaction

(20) Diastase reaction (iodine reaction): fairly strong

Enzymatic zone: 7 ± 2 mm on starch agar (Waksman's A) incubated in 10 days

(21) No cellulase reaction (cellulose.....filter paper)

(22) Nitrite production from nitrate on modified synthetic solution: somewhat unstable; generally positive, sometimes negative

(23) Carbon utilization: lactose, fructose, and xylose utilized; sucrose and inositol, uncertain; rhamnose, mannitol, and raffinose unutilized

(24) Habitat: soil

III. Consideration

Though this strain forms white and cottony aerial mycelium on most media, it does not show any remarkable character about the production of soluble pigments and the colors of substrate mycelium.

Consequently, there are many species which resemble apparently to this strain in some points.

Now this strain is compared with the following several species which resemble to this morphologically, especially in respect to spiral and whirl formation.

(1) *Streptomyces viridoflavus* Waksman and Taber (6)

Str. viridoflavus shows the following characters. "Vegetative growth: abundant lichenoid growth on most media; color of growth yellow-green, turning olive-green to almost brown....."

Aerial mycelium: Aerial hyphae formed in fascicles; greenish yellow in color, turning gray. Tendency to lose property of producing aerial mycelium. Tufts, with some curling of tips produced on certain media. On glucose asparagine agar, spore produced in chains, in whirls." (6)

By the facts that *Str. viridoflavus* forms a single spore at the end of the submerged

sporulating lateral branch and my strain (No. 508) does not form one, and the color of aerial and substrate mycelium of *Str. viridoflavus* differs from that of No. 508. Thus, they are distinctly different from each other.

(2) *Streptomyces circulatus* (Krassilnikov) Waksman and Lechevalier (2), (6).

The description of *Str. circulatus* is so brief that it is impossible to compare sufficiently with other species. But generally this strain grows more luxuriantly on synthetic medium than on organic media. For example, on nutrient agar the growth of colony is weak and on synthetic agar, good; while No. 508 shows good growth on both media. Moreover there are some differences on morphological characters as the following table.

	Whirl	Conidia	Sucrose inversion
<i>Str. circulatus</i>	Anitella-type	cylindrical~oblong 1.5 μ by 0.7 μ	negative
No. 508	Nitella-and Anitella-type	spherical~somewhat elliptical 0.7 μ ~0.8 μ by 0.8 μ	positive

(3) *Streptomyces rubr-reticuli* (Waksman) Waksman and Henrici (1), (5), (6)

This species forms many typical whirls and spirals on various media. The color of the aerial mycelium shows Rose to pink series on many synthetic media, while No. 508 is white. Moreover this species shows good growth on cellulose, while No. 508 does not.

(4) *Streptomyces reticuli* (Waksman and Curtis) Waksman and Henrici (1), (5), (6)

Str. reticuli forms white and cottony aerial mycelium on synthetic agar, and consequently this is one of the most similar species to No. 508 by naked eyes and forms whirls and spirals on glucose agar, but no spiral on synthetic agar.

Further, differences between *Str. reticuli* and No. 508 on cultural observation are as follow:

Media	Ca-malate agar	Glucose agar	Starch agar	Glucose broth	Glucose broth	Reaction of milk
	color of aerial mycelium	"	"	"	soluble pigment	
<i>Str. reticuli</i>	naphthalene yellow	"	lavender	brown	brown	unchanged
No. 508	white	"	white~brownish white	white	none	alkali

Followingly, *Str. reticuli* is distinct from No. 508.

(5) *Streptomyces albireticuli* Nakazawa (3)

Though this species forms white and cottony aerial mycelium, and many typical primary and secondary whirls on various synthetic media, and no spiral can be seen.

(6) *Streptomyces albus* (Rossi Doria emend. Krainsky) Waksman and Henrici (1), (5), (6)

Str. albus forms white aerial mycelium and spirals on several synthetic media and resembles to No. 508 remarkably by naked eyes' observation. But on microscopical observation, the former does not form any whirl at all. And moreover haemolytic reaction of *Str. albus* is negative, while No. 508 is positive.

Because of the results above mentioned, the author has decided No. 508 to be a new species and named it *Streptomyces spiroverticillatus*.

This specific epithet means that this forms spirals and whirls and it is an intermediate type between Nitella- and Anitella-type. (4)

Summary

Two new strains (No. 507 and No. 508), isolated from the soil collected respectively at Higashinoshiro City (Akita Prefecture) and at Kuchian Station (Hokkaido) in July 1955, were identified to be a new species, *Streptomyces spiroverticillatus*, basing on morphological and cultural studies. The specific epithet shows the characters forming spirals and whirls on the aerial mycelium.

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Observations on the Annual Growth Cycle of Marine Algae on a Reef at Manadzuru on the Pacific Coast of Japan

by Shigetoshi MATSUURA*

松浦茂寿*: 真鶴崎の一片礁における海藻発生の周年変化

Received December 25, 1957

Introduction

In spite of a multitude of published works chiefly on the classification and distribution of marine algae in this country, there are few presenting the records of researches pertaining to the ecological features of algal growth (1, 2, 3). Even more scanty are the papers that have reported the regular observations made from the point of view of plant sociology, and with special reference to the annual cycle of algal flora at a certain,

* Born 25th April, 1898; deceased 10th September, 1957, at Odawara, Japan, after thirty years of untiring teaching at Odawara High School and life-long devotion to research on the marine algae of Sagami Bay and on the land flora of the Hakone district.

"fixed" location on the sea coast of Japan. It is the purpose of this paper to publish the results of regular observations carried out by the present author at the "observation reef" he has defined at Manadzuru Peninsula, Kanagawa (Province of Sagami). The regular inspection of the place commenced in November, 1937 and was carried out continually until the next October. During this period, the author made it a rule to visit the place at least twice a month, taking every chance of the major tides for studying every aspect of algal growths above and below sea level. From the reef point under investigation, as many as 88 species among the 126 so far discovered in the vicinity (4, 5, 6)* have been identified, belonging to the families of Chlorophyceae, Phaeophyceae, and Rhodophyceae. The seasonal changes in the algal growth are presented in a table in terms of appearance, vigor and decay of individual algal species. The results obtained are briefly discussed from a plant sociological point of view.

General Aspects of Environment



Map. I. Manadzuru Peninsula

Location Manadzuru is a peninsula of about three kilometres in length, extending SES into Sagami Bay, with an average height of 50 to 90 metres above sea level. Most of the land is covered with rich growths of deciduous trees. Although there is a coastal fishery harbour at its northern neck, the greater part of the peninsula remains uninhabited (see Map I). The observation station for this study was chosen from among the many

* These comprise the following 45 species of algae;

CHLOROPHYCEAE; *Cladophora writhtiana* Harvey; *C. rudolphiana* (Ag.) Harvey; *Codium cylindricum* Holmes; *C. divaricatum* Holmes; *C. latum* Suringar; *Enteromorpha compressa* (L.) Greville var. *intestinalis*.

PHAEOPHYCEAE; *Pachydiction coriaceum* (Holmes) Okamura; *Sargassum pilulifera* C. Agardh; *S. horneri* (Turn.) C. Agardh; *S. gigantefolium* Yamada; *S. tortile* C. Agardh; *Spathoglossum variabile* Fig. et DeNot; *Undaria pinnatifida* (Harvey) Suringar; *Zonaria diessingiana* J. Agardh.

RHODOPHYCEAE; *Aeodes lanceolata* Okamura; *Ahnfeltia concinna* J. Agardh; *A. furcellata* Okamura; *A. paradoxa* (Sur.) Okamura; *Amphiroa zonata* Yendo; *Bangia fusco-purpurea* (Dill.) Lyngb.; *Callophyllis crispata* Okamura; *Cauleanthus Okamurai* Yamada; *Centroceras clavulatum* (Ag.) Mont.; *Ceramium japonicum* Okamura; *Champia bifida* Okamura; *Delisea pulchra* Montagne; *Dudrenaya japonica* Okamura; *Galaxaura falcata* Kjellman; *Gelidium linoides* Kuetzing; *G. subcostatum* Okamura; *Gracilaria confervoides* (L.) Grev.; *G. chorda* Holmes; *G. incurvata* Okamura; *Grateloupia ramosissima* Okamura; *G. turuturu* Yamada; *Hypnea cervicornia* J. Agardh; *Liagora lepra* J. Agardh; *Lithophyllum canescens* Foslie; *Martensia denticulata* Harvey; *Meristotheca papulosa* (Mont.) Agardh; *Nemalion vermiculare* Suringar; *Procamium telfairiae* Harvey; *Polysiphonia crassa* Okamura; *Porphyra tenera* Kjellman; *Sarcodia ceylanica* Harvey.

reefs that enclose the Shikkake inlet, which is situated on the southern side near the base of the peninsula. The theodolitic location of the place reads as follows;

Long. 139° 8' 30" E Lat. 35° 8' 45" N.

The area of the "observation reef" is about 87 m² (above mean sea level).

Climatic conditions According to the data published by Kanagawa Prefecture covering the period from 1935 to 1938, the annual atmospheric conditions at Manadzuru are as follows:

Average maximum temperature		18.6°
Average minimum temperature		11.8°
Maximum temperature (absolute maximum)		33.2°
Minimum temperature (absolute minimum)		-4.4°
Rainy days;	146	Snowy days; 7
Frosty days;	13	Stormy days; 39
Total precipitation (rain & snow)		2091.1 mm

Figure 1 shows temperature and precipitation data for the months of the year (Kanagawa Prefecture; Meteorological Station).

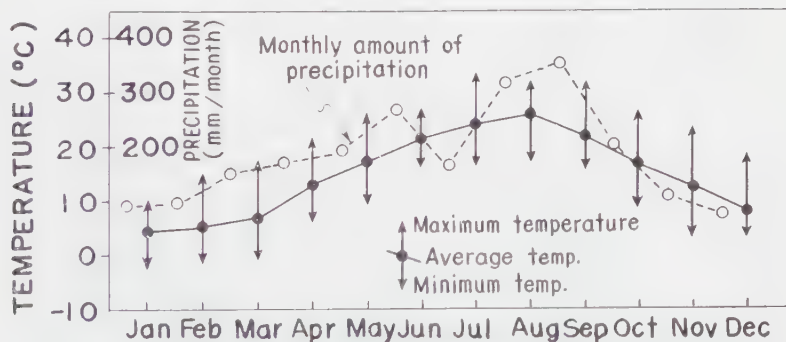


Fig. 1. Atmospheric temperature and precipitation data for the months of the year (Kanagawa Prefecture Meteorological Station).

Conditions of sea current and water A branch of the main warm current, the "KUROSHIWO", which strikes against Jôgashima, runs along the west coast of Miura Peninsula, and then past Enoshima, almost disappears off the mouth of the Banyû River. Manadzuru is therefore not subjected to the direct influence of the Kuroshiwo current. A counter current, however, streaming in the opposite direction along the shore of the Bay of Sagami, sweeps the side of the peninsula under investigation in a westwards direction. Figure 2 presents data for the annual changes in sea water temperature at the sea surface and at a depth of 25 m below the surface, as observed at the Kanagawa Prefecture Fishery Experimentation Station, (1935-1939), which is situated not very far from the "observation reef" under study. At the sea surface, the temperature was lowest in February (13°), and highest in August (25°), whereas at a depth of 25 m below sea surface the minimum was observed in March (14.5°) and the maximum in September (23°). Data for atmospheric temperature are also shown in the same figure,

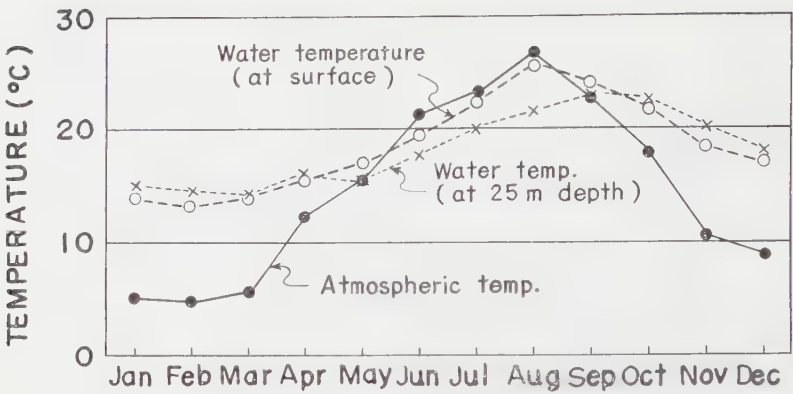


Fig. 2. Sea water temperature for the months of the year (Kanagawa Prefecture Fishery Experimentation Station (1935 1939).

with a maximum and a minimum almost coinciding in time with the respective temperature characteristics at the sea surface. The specific gravity of the sea water at the Experimentation Station has been reported to be as follows;

	Surface	25 m depth	Average
January	1.02564	1.02563	} about 1.025
March	1.02566	1.02563	
June	1.02485	1.02544	
Augst	1.02423	1.02500	

The transparency measured as usual using a white plate of 30 cm diameter;

	Jan.	Mar.	May.	Jul.	Sept.	Nov.
Transparency (metres)	15	16	21	6	12	5

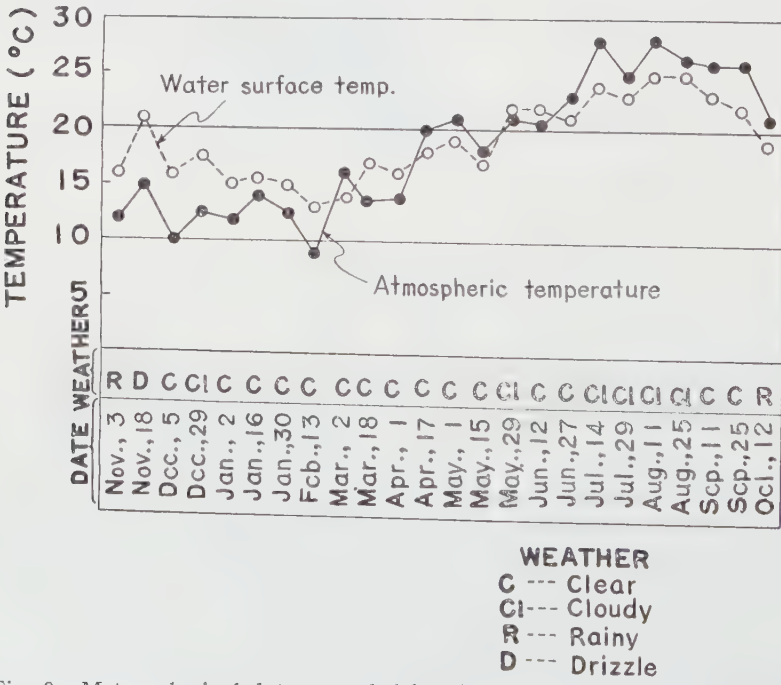


Fig. 3. Meteorological data recorded by the author on inspection of the observation reef.

The figures occasionally obtained by the present author on his inspection of the observation reef are presented in Figure 3.

“*Observation reef*” The *observation reef* to be investigated was chosen by the author, after several years of preliminary studies around the neighbouring sea coast, be-



Map. II. Observation reef.

Notice In Map II and Table I the following abbreviations are used.

HP	Highest point of the reef	220 cm	Upper zone
ES	Highest line of equinoxial spring tide	80 cm	
US	Upper line of average spring tide	60 cm	
UN	Upper line of average neap tide	20 cm	
M	Mean level	0 cm	
LN	Lower line of average neap tide	-20 cm	Middle zone
LS	Lower line of average spring tide	-60 cm	
LES	Lower line of equinoxial spring tide	-80 cm	
PD	Permanently dipped zone	below -80 cm	Lower zone
DP	Deepest part of the area under investigation	-180 cm	

(c; Cystocarps, t; tetraspores)

Months of the year

Vertical Distribution

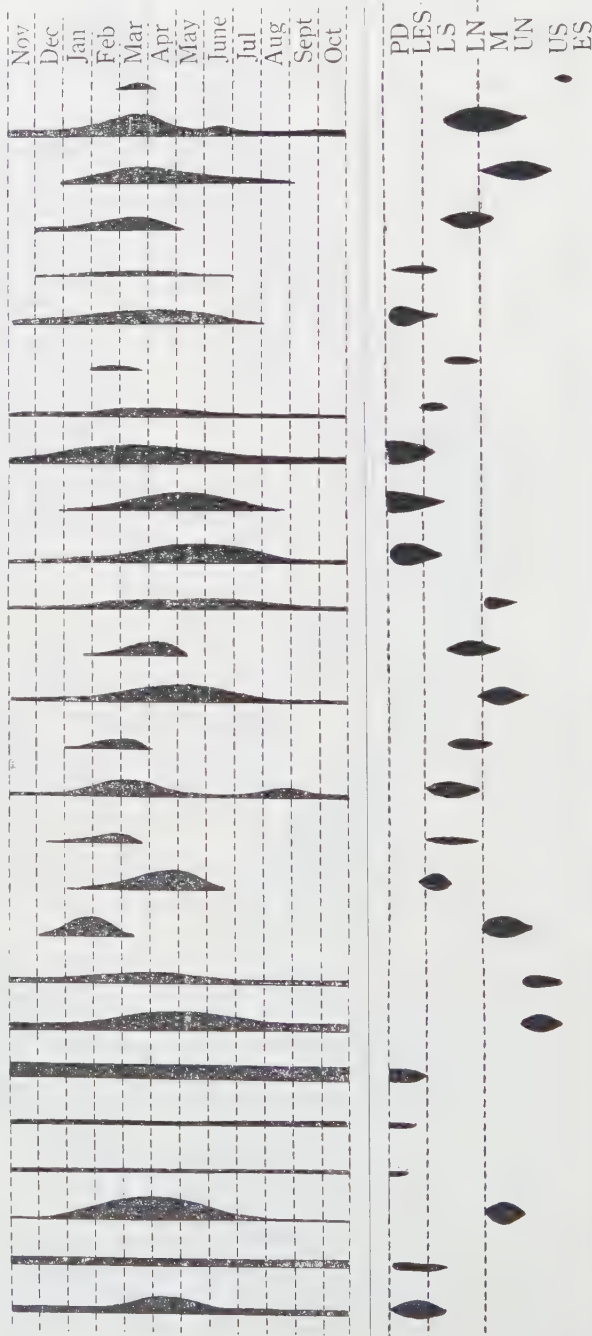
Lower zone

Middle zone

Upper zone

PHAEOPHYCEAE

10. *Dictyota dichotoma* (Huds.) Lamouroux
11. *Padina arborescens* Holmes
12. *Petrospongium rugosum* (Okamura) Setchell et Gardner
13. *Leathesia difformis* (Linne) Areschoug
14. *Myelophycus caespitosus* (Harvey) Kjellman
15. *Scytosiphon lomentarius* (Lyngbye) J. Agardh
16. *Colpomenia sinuosa* (Roth) Derbes et Solier
17. *C. bulbosa* (Saunders) Yamada
18. *Hydroclathrus clathratus* (Bory) Howe
19. *Eudarachne binghamiae* J. Agardh
20. *Ishige okamurai* Yendo
21. *I. foliacea* Okamura
22. *Eisenia bicyclis* (Kjellman) Setchell
23. *Ecklonia cava* Kjellman
24. *Cystophyllum sisymbrioides* J. Agardh
25. *Hizikia fusiforme* (Harvey) Okamura
26. *Sargassum patens* C. Agardh
27. *S. serratifolium* C. Agardh



	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sept	Oct	PD	LFS	LS	LN	M	UN	US	ES				
28. <i>S. ringgoldianum</i> Harvey																								
29. <i>S. sagamianum</i> Yendo																								
30. <i>S. thunbergii</i> (Mertens) O. Kuntze																								
31. <i>S. hemiphyllum</i> C. Agardh																								
RHODOPHYCEAE																								
32. <i>Porphyra suborbiculata</i> Kjellman																								
33. <i>P. dentata</i> Kjellman																								
34. <i>Nemalion pulvinatum</i> Grunow																								
35. <i>Scinaia japonica</i> Setchell																								
36. <i>Gloiophloea okamura</i> Setchell																								
37. <i>Galaxaura fastigiata</i> Decaisne																								
38. <i>Actinotrichia fragilis</i> (Forsskal) Boergesen																								
39. <i>Gelidium divaricatum</i> Martens																								
40. <i>G. pusillum</i> (Stackh.) Le Jolis																								
41. <i>G. pacificum</i> Okamura																								
42. <i>G. japonicum</i> (Harvey) Okamura																								
43. <i>G. amansii</i> Lamouroux																								
44. <i>Pterocladia tenuis</i> Okamura																								
45. <i>Acanthopeltis japonica</i> Okamura																								
46. <i>Chondrococcus hornemanni</i> (Mertens) Schmitz																								
47. <i>Amphiroa zonata</i> Yendo																								
48. <i>A. ephedraea</i> Decaisne																								
49. <i>A. declinata</i> Yendo																								
50. <i>Corallina pilulifera</i> Postels et Suprecht																								
51. <i>Jania unguolata</i> Yendo																								
52. <i>Grateloupia filicina</i> (Wulfen) J. Agardh																								
53. <i>G. carnosa</i> Yamada et Segawa																								
54. <i>G. prolongata</i> J. Agardh																								
55. <i>G. livida</i> (Harvey) Yamada																								
56. <i>G. Okamura</i> Yamada																								
57. <i>G. imbricata</i> Holmes																								
58. <i>G. elliptica</i> Holmes																								
59. <i>Polyopes polyideoides</i> Okamura																								

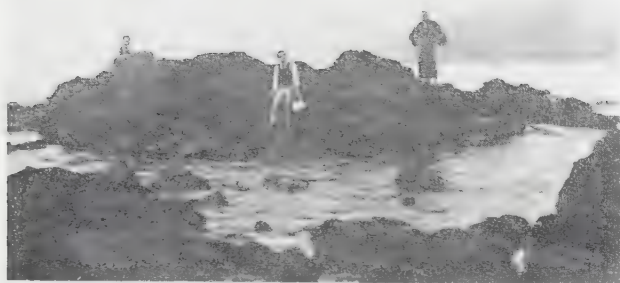


Fig. 4. The view of the observation reef from NE. (Author in center)

ever, the breaker at the front of the reef measured a height of one to two metres, and in stormy weather even waves as high as three metres were not very rare. At high tide the whole surface of the reef was subject to the action of waves even in moderate weather. At neap tide splashes scarcely reached to the higher zones of the reef, exposing the surface of the rock to heat and dessication in fine weather.

Lithological aspects The rocks of the reef consist of two-pyroxine andesites from Hakone Volcanic Lava, a branch streaming from the ancient somma of Hakone into the sea about the middle of Diluvium. It is also likely that the geological features of the coast in question have been significantly modified by successive elevations, some of which have occurred even in relatively recent periods.

Results

Annual cycle of algal succession The results obtained as regards the annual cycle of algal growth on the *observation reef* are summarized in Table 1. The width of the black area represents the relative vigor of growth of each algal species indicated. The marks *c* and *ct* in the same table indicate, respectively, the formation of cystocarps and tetraspores on the thallus. The right half of the table shows, in addition, the zone of the reef dominated by each individual species. Negative signs before the figures in this part of the table represent the depths below mean sea surface. (For abbreviations ES, US etc., see the notice on page. 97)

Seasonal changes in number of algal species The changes in number of algae discovered each month on inspection of the reef are illustrated in Figure 5 and summarized in Tables 2A and 2B. These results may be abstracted as follows.

i) The total number of algal species to be discovered on this reef is at its minimum of 39 in autumn (Sept.~Nov.), rising gradually to a maximum of 88 in the period from March to May. A gradual decrease then follows during the summer to the above-mentioned minimum of 32 in October.

cause of the ease of approach and especially because of the variety in coastal formation which gave rise to the richness in algal flora as stated above. The surface of the reef under investigation is oblong in shape, about 8.5 m wide and about 12.5 m long, with a total area of about 87 m² above mean sea level (see the contour Map II and the photograph in Fig. 4).

Waves In calm weather, the agitation by waves was almost negligible. More usually, how-

Table 2A. Monthly changes in number of algae.

Division	Month												Total
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sept.	Oct.	
Chlorophyceae	3	6	7	8	9	8	8	6	5	4	4	3	9
Phaeophyceae	16	18	22	22	22	19	19	18	17	17	17	16	22
Rhodophyceae	14	18	32	41	54	56	55	50	45	30	17	13	57
Total	33	42	61	71	85	83	82	74	67	51	38	32	88

Table 2B. Seasonal changes in number of algae.

Division \ Season	Season			
	Sept.—Nov.	Dec.—Feb.	Mar.—May.	Jun.—Aug.
Chlorophyceae	5 (56%)	8 (89%)	9 (100%)	6 (67%)
Phaeophyceae	16 (73%)	22 (100%)	22 (100%)	18 (82%)
Rhodophyceae	18 (32%)	45 (79%)	57 (100%)	50 (87%)
Total	39 (44%)	75 (85%)	88 (100%)	74 (84%)

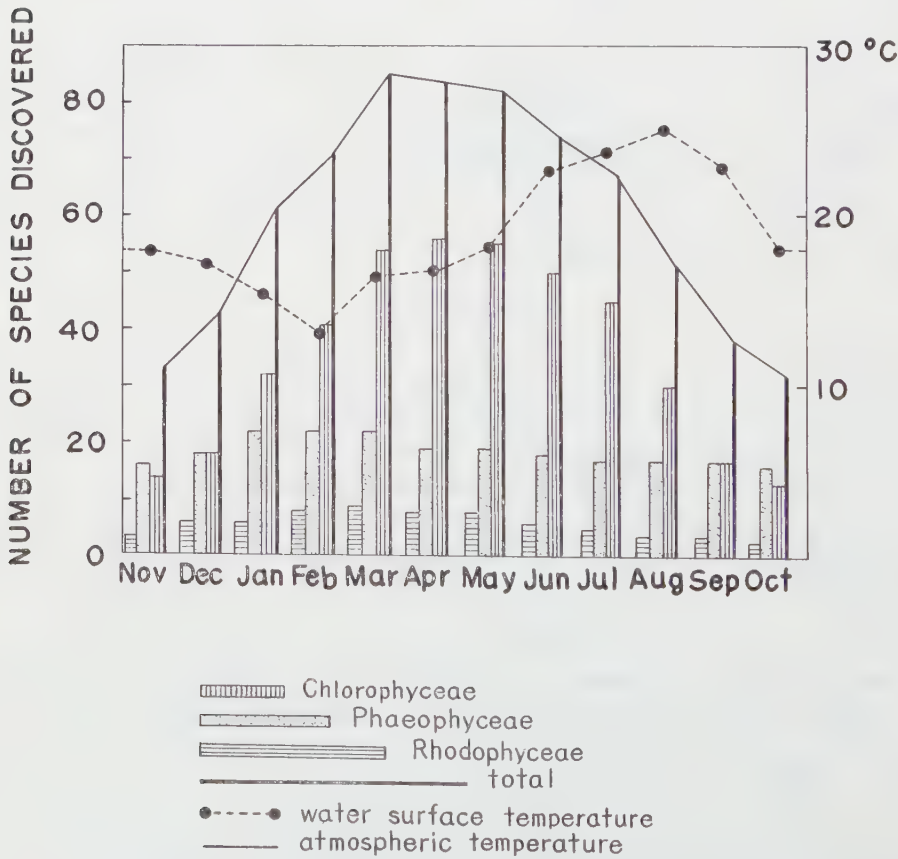


Fig. 5. Number of algal species discovered each month on the observation reef.

ii) Another remarkable feature in this respect is the dominance of red algal forms in the total flora throughout the seasons of the year, the rise in the total number of algal species being largely dependent on this particular group. The extent of the fluctuation in the number of Phaeophyceae species was relatively small, most of them being perennial forms. Green alga flora was found to be not very rich so far as the number of species was concerned. Eight to nine species were identified during the months from February to May, when they showed most prolific growth.

In summary, throughout the seasons of the year, of the total of 88 species discovered on the observation reef, 57 (65%) belonged to Rhodophyceae, 22 (25%) to Phaeophyceae, and 9 (10%) to Chlorophyceae. It should be kept in mind that the above list may lead to an estimation somewhat too low for the actual number of algal species involved. There are species of algae missing from the table, that, nevertheless, have been collected by the present author, during the said observation period as well as in more recent years of extended survey, from the neighbouring areas around the observation reef. All of them, however, being found to be forms of rather rarer occurrence and also of less abundant growth, the above estimation for the number of the algal species involved seems to the present writer to be fairly reliable. The records for the growth periods of the individual algal forms also might have been modified to some extent if we could have extended our investigation to cover the whole surface of the Manadzu-ru area, since there was naturally no reason whatsoever that every algal species should have appeared earliest and disappeared latest on this particular reef point. Here also, the year-long observation of the present author indicates that the conclusion to be drawn from the above-presented figures may not be very significantly different from the real picture of algal succession in this part of the sea coast of Japan.

iii) From the results shown in Figure 5, it is apparent that the time of increasing appearance in number of algae coincides with the cold season, while the period of marked disappearance occurs in the hot season. This is a common knowledge among our algologists, and it leads them to correlate the fact directly with the annual cycle of temperature variation. The present author, however, is rather inclined to restrain such conclusion until more direct experimental evidence is furnished as to the actual effect of temperature on the growth and decay of sea weeds. It will perhaps be more fruitful to present figures correlating the counts of algal species, on the one hand, with those for first appearance, vigor of growth, and last disappearance, on the other hand, as well as those pertaining to the formation of cystocarps and tetraspores in red alga forms in particular. (Figures 6 and 7). From these results it is safe to conclude that the annual cycle of algal growth on the reef is initiated in the months Dec-Jan-Feb-Mar, when the largest proportion of the inhabitants make their first appearance on the reef. The abundance of algal species attains a maximum in the month of March, new comers becoming rather scarce in number after this time. Most species show the highest vigor of vegetative growth in May, when the sea flora becomes therefore richest both in number and luxuriance of growth. With most species of Rhodophyceae, the group occupying the

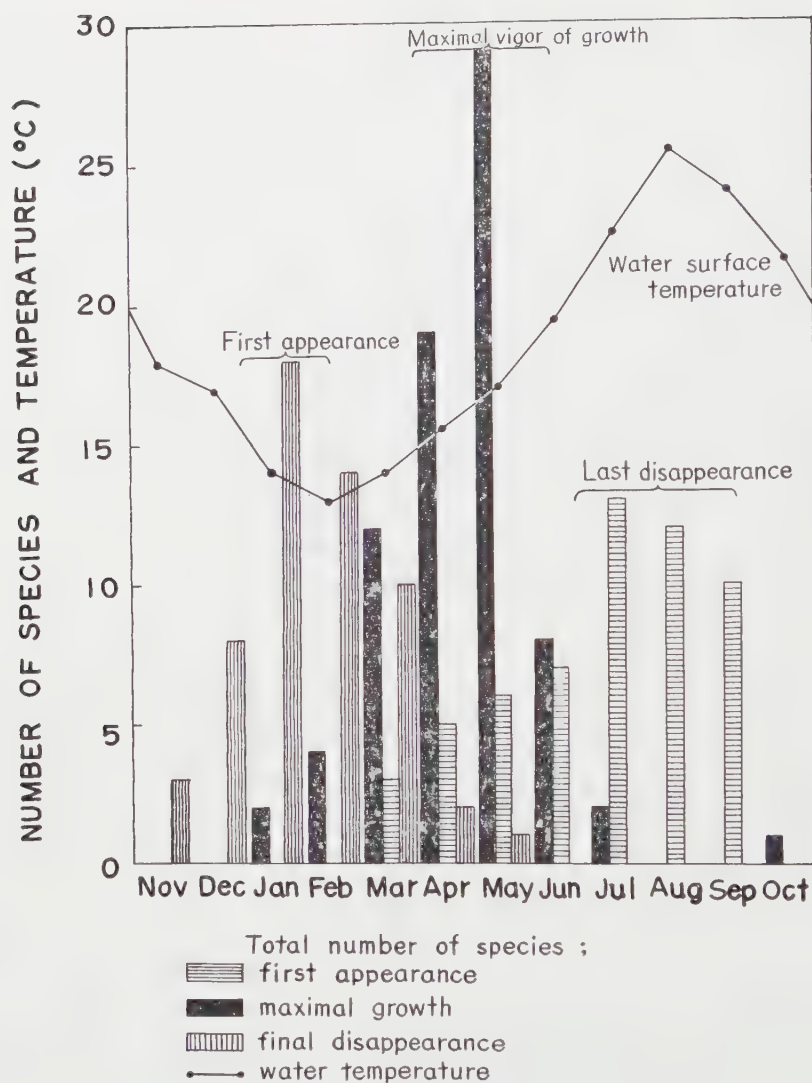


Fig. 6. Number of first appearance, maximal growth, and final disappearance of algae in each month of the year.

dominant place in the algal floral under investigation, the season of maximal vigor is the season of spore formation (cystocarps and/or tetraspores) or the time immediately preceding it (See Fig. 7). Then follows a period of decay and finally the time for their temporary disappearance from the reef, the number of disappearing species being most numerous in the months July-Aug-Sept, when the view on the coast is most desolate to the eyes of an algologist.

There are, in addition, more durable forms of algae persisting throughout the year. The number and the percentage of perennial algae were found to be as follows; 3 perennials out of the total of 9 Chlorophyceae (33%); 16 of 22 Phacophyceae (72%); 13 of 57 Rhodophyceae (23%).

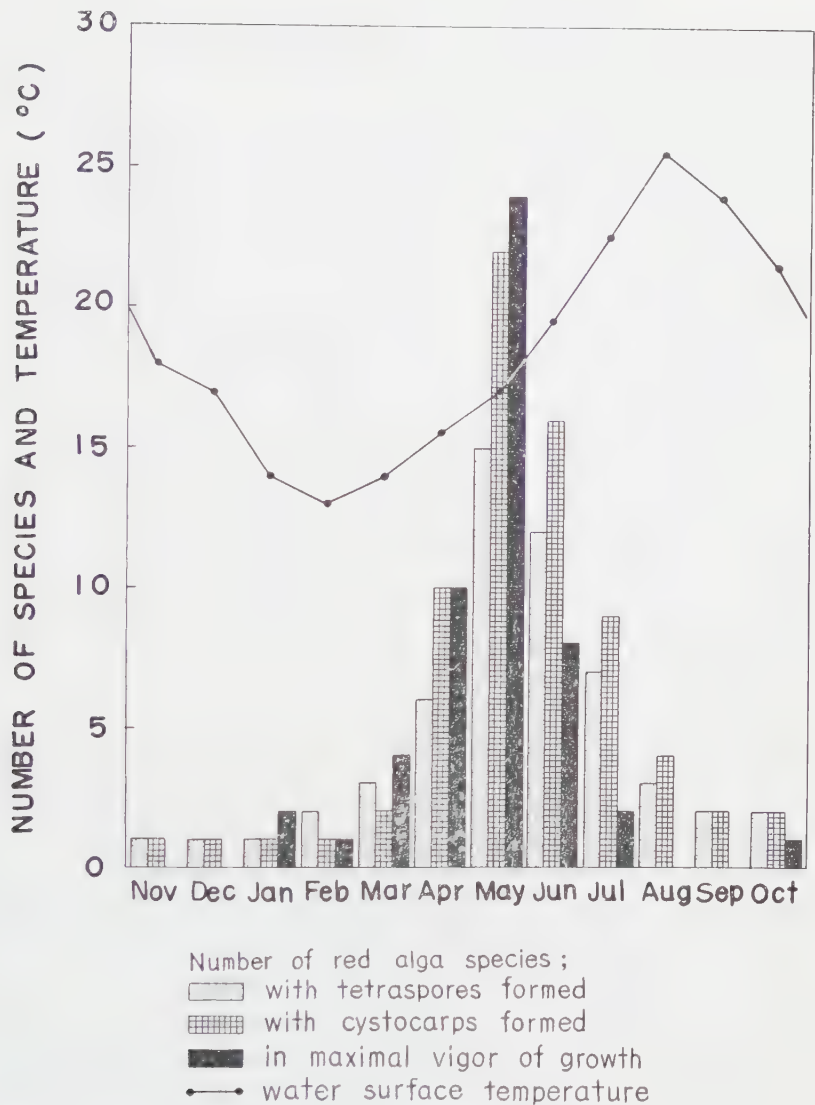


Fig. 7. Monthly changes in number of red alga species carrying tetraspores or cystocarps.

Vertical distribution of the algae Turning our eyes to the vertical distribution of the algae, the results of the observations will be summarized as follows (Table 3).

Table 3. Number of species discovered in various zones of the reef.

Division Zone	Chlorophyceae	Phacophyceae	Rhodophyceae	Total
Upper	4 (29%)	9 (29%)	17 (22%)	30 (24.5%)
Middle	7 (50%)	11 (35.5%)	38 (49%)	56 (46%)
Lower	3 (21%)	11 (35.5%)	22 (29%)	36 (29.5%)

The number of the algal species was found to be largest in the middle zone, (from

20 to 80 cm below mean sea level; see above). About half the members of Chlorophyceae and Rhodophyceae are inhabitants of this part of the reef. In the case of Phaeophyceae, a more uniform distribution throughout various depths is to be noticed. The habitual way of considering the green, brown and red algae as the representative dwellers, respectively, of shallow, middle, and deep zones of the sea coast seems thus to be rather inappropriate, so far as the number of species characteristic of each zone is concerned. Roughly speaking, most of the perennial forms are deepwater inhabitants; out of 33 such species, 17 belong to the lower and 7 to the middle zone (see Table 4). It will

Table 4. Duration of growth period.

Months Zone	1~3	4~6	7~10	Perennial
Upper	1 (17%)	6 (22%)	6 (28%)	9 (27%)
Middle	4 (67%)	15 (56%)	8 (36%)	7 (21%)
Lower	1 (17%)	6 (22%)	8 (36%)	17 (52%)
Total	6	27	22	33

also be noted in this connection that in the middle zone of the reef, the number of species in the stage of their most vigorous growth reaches a maximum in the month of March, whereas in the lower zone there is a delay of about two months before that part of the flora attains its maximum (see Table 1).

Dominant species of algal flora *Gelidium divaricatum* and *Gigartina intermedia* are the two dominant species of the upper zone, covering most proportion of the area. *Chondrus ocellatus* also shows abundant growth in the upper zone. If we are to adopt the subdivision of the species into forma *canaliculatus* and forma *typicus*, it will be perhaps worth mentioning that the former is dominating the upper zone, especially the washed surface of the rock, while the latter is covering the lower part of the middle zone. There seems to be a rather gradual change according to the depth of water from the smaller sized *canaliculatus* form to the larger sized forma *typicus*. *Sargassum thunbergii* is the dominating species of the middle zone, often covering that part of the reef with thick rows of fully developed fronds. *Sargassum sagamianum* is another representative of the genus occupying considerable space in the middle and lower zones. It forms usually a densely packed colony, excluding all other algae from the circle, except for such subtle forms as *Hypnea japonica* which is capable of developing its own frond by clinging to the upper branches of other more stout forms of sea weeds. *Chondria crassicaulis* also is apt to form densely populated pure colonies of considerable extension in the middle zone of the reef. All the above mentioned dominant species, except *Chondria crassicaulis*, being perennial forms, the general feature of the algal vegetation around the reef is determined by the cycle of growth of these species throughout the year.

Algo-geographical Observation

The most prominent feature with the algal flora investigated is the dominance of warm current-forms. In fact, out of the total of 88 species discovered on the observation reef, as many as 82 belonged to the warm current-species. Six other species were ubiquitous forms being distributed throughout the seas from the tropic to the frigid zone. Among the 82 warm current-species mentioned above, 76 were typical temperate zone-inhabitants, while the other 6 were known to occur on the coasts of the temperate as well as the tropic seas.

The algal flora concerned included 28 typical Pacific coast dwellers (34%); the other 54 were the species known both from the Pacific as well as the Japan Sea coast of Japan. Almost half of the members of the Flora under investigation was indigenous to this country. In the above plant geographical consideration, reference has been made on the records of individual algal species as compiled by the late Professor K. Okamura (7) in his monumental work "The Algae of Japan" (1934)*. Additional evidence was also obtained from the occasional observations by the present author himself made around various places along the sea coast of this country from South-Sacchalene (once belonging to Japan), Hokkaido, the Main Island, Shikoku, Kyushuu to Ryuukyuu.

Concluding Remarks

Frankly stating the matter, the present author was surprised at the beginning of the present work, to discover how little had been known about the most common question, in what months of the year each species of sea weed first appeared, developed and finally disappeared. In spite of the generally recognized importance of marine algae from the stand points of fishery and marine agronomy, and notwithstanding the number of marine experimental stations distributed around the whole coast of this country, there had been scarcely a single record of systematic survey undertaken to reveal the actual features of the annual succession of marine algal flora at fixed points or definite regions of the sea coast. The labor of regular visits throughout the year to a definite observation point, following the appearances and decays of the commonest kinds of sea weeds, must have been too cumbersome a work for the busy university algologists and the official fishery bureau men. Most of the knowledge on the distribution of marine algae has been based on the compilation of records of collections by learned and amateur algologists who were usually interested in fully grown specimens of sea weeds suitable for herbarium preservation. Early stages of insignificant growths thus have escaped the scope of their observation. There have been, on the other hand, multitudes of untiring works of plant embryologists investigating the earlier stages of algal development. The studies of this category, however, are practically limited to a limited number of really representative forms of algal tribes and genera, and at the same time, are mostly concerned with the earliest stages of algal life. Although the method of the present study

* Various other papers were also consulted, including those indicated in Ref. 8~.

was mostly restricted to the observations with the naked eyes, and the search for the first appearance and the last remains of algal growths may be insufficient to be regarded as exhaustive, still it will be granted to the present author to profess that the results described above may not be very far removed from the real picture of floral succession under investigation. It is the opinion of the present author that the kind of general survey reported in the present study must also serve as the fundamental basis for the practice of marine agronomy, since it comprises the knowledge of growth of certain sea weeds of practical importance such as *Gelidium* and *Hizikia*, as well as information on other forms of marine algae that are in immediate competition with them.

It is really regrettable to the author that he had to postpone the more advanced and more interesting researches along this line of approach to the unpromised future, but he heartily hopes that the work will be continued by those who may be interested in the subject. He will be contented for the present to mention here the ideas he has been cherishing, although he could not put them into effect in his life time;

i) The same type of survey should be carried out simultaneously at different points along the entire coast of this country.

ii) More advanced investigation should be carried out with the purpose of analyzing the effects of various environmental factors such as depth, temperature, exposure to air, agitation by waves and especially, light intensity, as they co-operate in the formation and the succession of the algal flora.

iii) The use of artificial test blocks of various kinds of natural as well as synthetic materials submerged at various periods of the year at various parts and depths of the coast will serve in furnishing data on the potential productivity of the marine flora involved. The use of glass plates will be of great help in facilitating continual direct observation under the microscope, especially with the earlier stages of algal growths.

Summary

With the purpose of elucidating the annual cycle of algal flora at a certain "fixed" location on the Pacific coast, regular observations were carried out at the "observation reef" defined by the author at Manadzuru Peninsula, Kanagawa, Japan.

The results of the regular inspection of the place from November, 1937 to October, 1938 were described with respect to the number of algal species discovered at the observation reef, with special reference to the first appearance, the maximal vigor of growth and the final disappearance of each algal form.

Summing up through the year, there were 88 species of algae discovered from the observation reef, which belonged to the families of Chlorophyceae (9 species), Phaeophyceae (22 species) and Rhodophyceae (57 species).

The seasonal changes in the algal formation were investigated by following the life cycle of each algal form from its first appearance on the reef to its final disappearance from the place. Formation of cystocarps and tetraspores were also followed with the red alga forms.

The results of the study were discussed from the algo-geographical point of view.

Acknowledgements

The author wishes to thank Prof. Y. Yamada of Hokkaido University, Institute of Algal Research, for his interest and advice during this study. The writer's thanks are also due to the late Prof. K. Okamura for his invaluable help and infallible guidance in this field of algal research. He appreciates also the cooperation of his son, Seirô Matsuura, and one of his old students, Dr. Atusi Takamiya, in preparing and reviewing the manuscript. It is also a pleasure to acknowledge here the warmhearted help afforded to the author by his colleagues and friends during the course of the study.

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Vol. 71, No. 835, p. 5, line 14 from bottom, insert *through* between *elongated* and *the plasticization*.

On the Photosynthesis of Natural Phytoplankton under Field Conditions

by Shun-ei ICHIMURA*

市村俊英*: 自然状態における植物性プランクトンの光合成

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In the last decade, the problem in limnology has been focused upon the biological production. As all life in a lake depends directly or indirectly upon the organic matter synthesized by plants, the first step in the study of biological production in a lake should start from the photosynthesis of phytoplankton, on which the primary production is based. Regarding the photosynthesis of algae, numerous studies have been reported by many plant physiologists. However, they have concentrated their studies mainly on the analyses of mechanism of photosynthesis, and the experiments were performed with pure cultured algae under optimal condition in laboratory. On the contrary, the informations on photosynthesis of phytoplankton under natural condition are still inadequate for ecological elucidation of productivity of lakes. Therefore, for the ecological study of production of lakes, it is necessary to clarify the characters of photosynthesis proceeding in nature more quantitatively. The following study was undertaken to fill up these gaps.

Methods

Photosynthesis was measured by the "in situ" method. Sample water was taken up from the surface or various depths of a lake, then filled into clear and dark glass bottles of 100 ml. and finally the bottles were lowered down to the depth from which the water was taken. After certain hours, the increase or decrease of oxygen dissolved in the water of bottles was determined by Winkler's method. The amount of real photosynthesis was obtained by adding the respiration measured in the dark bottle to the net photosynthesis measured in the clear one. The suspension time did not exceed 6 hours in all cases (refer to Verduin, J. 1956, Ichimura and Saijo, in press). The total photosynthesis in a day was estimated as the double amount of a half day photosynthesis measured from noon to sunset. Density of phytoplankton in the water was determined by pigment analysis after filtration with two sheets of filter paper (Toyo, No. 101), and indicated as the chlorophyll amount (see Gessner, 1944, Hogeitsu and Ichimura 1954). As seen in Table 1, the photosynthetic capacity of filtrated water was so low and so greatly departed from the initial capacity of

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan. 東京教育大学理学部植物学教室

Table 1. Comparison of photosynthetic activity between raw water and filtrated water which was filtrated through two sheets of filter paper (Tōyō, No. 101) with a suction apparatus.

Lake	Dominant phytoplankter	Photosynthetic activity O ₂ mg./l/day	
		Raw water	Filtrated water
Jōnuma	<i>Chlamydomonas</i> sp.	1.075(1.00)	0.114(0.106)
Kasumigaura	<i>Microcystis</i> sp.	0.847(1.00)	0.082(0.097)
Teganuma	<i>Fragilaria</i> sp.	0.701(1.00)	0.046(0.066)

raw water that the phytoplankton which passed through the two sheets of filter paper may be less than 10 per cent of the total phytoplankton in the sample water. In the present study, the photosynthesis of phytoplankton is expressed by the amount of oxygen evolved per mg. chlorophyll corrected for 10 per cent error.

Results

A. Daily and vertical change in the rate of photosynthesis.

Variation of illumination in a day affects on the photosynthetic rate remarkably. Fig. 1 shows the characteristic feature of the change of photosynthetic rate at various depths during the course of a day. Data were obtained at Lake Teganuma on a clear day in May. The transparency measured with Secchi's disc was about 1.3 metres. The material used for this experiment was collected with a plankton net from surface of the lake and its photosynthesis was measured at various depths of the lake.

In the morning hours the highest rate was measured at the surface, but in midday that was obtained at deeper depth, because the photosynthesis in the upper layer was interfered by too intense sun light. The highest rate again shifted at the surface in the afternoon. These photosynthesis curves with two peaks are similar to that described by Jenkin (1937), and resemble to those obtained in land plants. Of course, the form of photosynthesis curves obtained at each depth differs from each other according to the light intensity prevailing on the lake surface and the transparency. For instance, the photosynthesis curve obtained at the surface on cloudy day did not show two peaks. Table 2 indicates the variation of the daily total amount of photosynthesis with increasing water depth. The maximum rate was generally found at some depth below the surface on clear day, and on cloudy day it shifted upwards. In winter, solar illumination is so low that the maximum rate was always found at the surface. The direct relationship between light intensity and photosynthetic rate in lake was discussed with the data of light intensity measured photoelectrically at

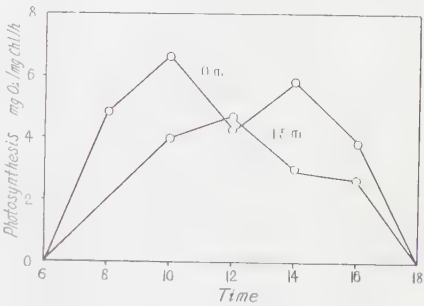


Fig. 1. Daily change in photosynthetic rate of phytoplankton in Lake Teganuma.

Table 2. Photosynthesis rate of phytoplankton at various depths measured in various seasons. The value is expressed by O₂ mg. per chlorophyll mg. per day.

Lake Weather Month	Kasumigaura Cloudy		Nakanuma Sunny		Suwa Sunny		Teganuma Sunny	
	Sept.	Nov.	June	Aug.	Sept.	Dec.	Nov.	Feb.
Depth								
0 m	36	15	37	21	30	18	4	12
1 "	7	3	67	26	23	10	7	9
2 "	3	2	79	15	10	5	3	—
3 "	2	—	59	8	7	4	—	—
4 "	—	—	16	3	2	—	—	—

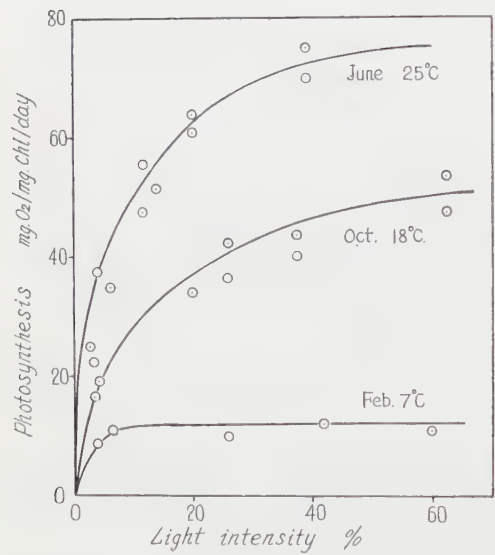


Fig. 2. Relation between photosynthesis of phytoplankton and light intensity measured under field condition.

various depths, at which the bottle was suspended. Results obtained at Lake Nakanuma in several seasons are shown in Fig. 2. The light-photosynthesis curve is similar to the well-known light photosynthesis curve in algae which has been obtained in laboratory by many investigators. The light saturation of photosynthesis was found at about 50 per cent of initial illumination. Further increase of illumination caused little change or even inhibition in photosynthetic rate.

The photosynthesis of phytoplankton showed considerable variation in the course of year. The results gained at Lake Nakanuma during 1950-1951, and those gained at Lake Teganuma during 1956-1957, are shown in Fig. 3. Measurement were performed two or three times a month, and the monthly mean of photosynthesis maximum in each "in situ" experiment was taken. The photosynthetic rate of phytoplankton obtained at Lake Nakanuma showed two marked pulses in early summer and in autumn. Namely, the photosynthetic activity began to increase on March and rapidly reached a large early summer value, and thereafter decreased during summer. It, however, increased again in early autumn and reached a large autumn value. During winter the photosynthetic rate exhibited very low or negative value. The feature of the curve obtained at Lake Teganuma, which is very shallow water and where higher water plants are vigorously growing, accord-

B. Seasonal change in rate of photosynthesis

The photosynthesis of phytoplankton showed considerable variation in the course of year. The results gained at Lake Na-

ed with that of Lake Nakanuma, but the extreme increase in autumn did not appear. It is interesting to note that the photosynthetic activity is roughly propotional to the amount of solar energy falling on the surface of lake. However, in a few cases exceptionally high photosynthetic activity in winter was observed, for example, at Lake Suwa in December (Hogetsu and Ichimura 1954), and similar phenomena were reported by Verduin (1956), too. These differences are obviously attributed to water temperature, light condition, nutrient concentration, character of algae, etc., in each lake. The author will touch these phenomena in other paper.

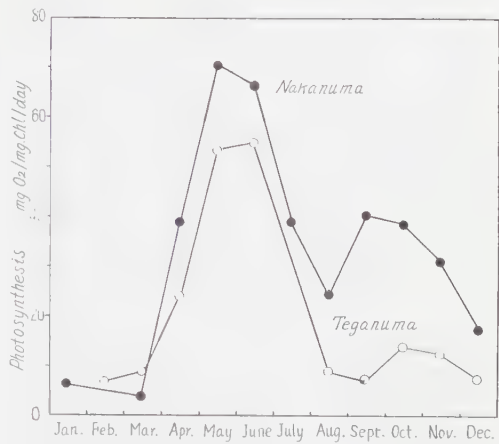


Fig. 3. Seasonal change in photosynthetic rate. Data in the diagram were obtained at 1 metre depth in each lake.

C. Maximum photosynthetic activity of phytoplankton under field condition

It is difficult to determine the maximum photosynthetic rate under natural condition, as the photosynthesis is limited not only by the external factors but also by the internal ones. The maximum rate in this work represents only the average of high values measured in the course of a year. The highest values obtained at the depth of optimal light intensity in various lakes summarized in Table 3. Although

Table 3. Maximum photosynthetic rate of phytoplankton measured in the course of year at several lakes. Photosynthesis was expressed by O₂ mg. per chlorophyll mg. per day.

E: Eutrophic lake, M: Mesotrophic lake, O: Oligotrophic lake

Lake	Date	Photosynthetic rate O ₂ mg./chl. mg./day	Dominant phytoplankter
Jōnuma(E)	Oct. 27, 1956	112.8	<i>Chlamydomonas</i> sp,
Suwa(E)	Oct. 18, 1954	105.0	<i>Microcystis</i> sp.
Kasumigaura(E)	Nov. 23, 1956	95.7	<i>Microcystis</i> sp.
Nakanuma(E)	June 5, 1952	76.9	<i>Fragilaria</i> sp.
Ushikunuma(E)	June 20, 1954	67.0	Desmid
Teganuma(E)	July 15, 1954	65.2	<i>Melosira italica</i>
Shinseiko(M)	July 11, 1956	44.4	Chlorophyceae
Yamanakako(M)	Sept. 8, 1956	42.0	<i>Botoryococcus</i> sp.
Akagi-Onuma(O)	July 11, 1955	25.0	<i>Asterionela</i> sp.
Ashinoko(O)	July 22, 1957	19.0	<i>Melosira</i> sp.

phytoplankton in extremely eutrophicated lake or in cultured algae sometimes showed higher rate than 100 mg O₂/chl. mg./day, natural phytoplankton in eutrophic lakes seems generally to show a somewhat lower rate than this.

The average maximum rate was situated between 80 mg and 90 mg O₂/chl. mg./day in eutrophic lake. 40 mg., and 50 mg. in mesotrophic lake, and 10 mg. and 20 mg. in oligotrophic lake. Because of lack of data on the diurnal change of photosynthetic rate, the maximum rate of per hour cannot sufficiently be indicated in this paper. For the purpose of reference, a few results were indicated in Table 4, in which

Table 4. Maximum photosynthetic rate of phytoplankton per hour measured at some lakes.

Lake	Date	Photosynthetic rate O ₂ mg./chl. mg./hr.	Dominant Phytoplankter
Jōnuma	Sept. 18, 1956	8.2	<i>Euglena</i> sp.
Kasumigaura	Sept. 21, 1956	7.2	<i>Microcystis</i> sp.
Nakanuma	May 30, 1957	7.7	<i>Eudorina</i> sp.
Teganuma	April 25, 1956	6.8	<i>Melosira</i> sp. <i>Eudorina</i> sp.
Kawaguchiko	June 28, 1955	4.6	<i>Melosira</i> sp.
Ashinoko	June 24, 1957	1.7	<i>Melosira</i> sp.

the data represent the maximum value in the course of a day. Though the photosynthetic rate was measured with various species taken from eutrophic lakes, those algae showed similar photosynthetic activity per unit chlorophyll, and 7.8 mg. O₂/chl. mg./hr. may be considered as an average maximum rate under natural condition. This value is well accordance with the results reported by other authors (Manning and Juday 1941, Gessner 1944). It is noticeable that the maximum photosynthetic rate based on unit chlorophyll falls nearly same values without regard to difference of phytoplankton species.

Therefore, the difference of photosynthetic activity in different species may sometimes be negligible with some limitation, if the photosynthetic rate is discussed on the basis of unit chlorophyll. These things have also been confirmed through experiments with various cultured algae (Ryther 1956, Ichimura, in press).

D. Relation of the photosynthetic capacity of lake water to the phytoplankton concentration in the water

In recent years, Hogetsu and Ichimura (1954) and Ryther (1957) performed the computation of the primary production from chlorophyll and light data. Assuming a strict proportionality between the photosynthetic capacity and the chlorophyll amount, it may be possible to deduce the primary production of phytoplankton population from the latter. Concerning this, an experiment was carried out. A part of the phytoplankton collected from water near surface with a plankton net was suspended in various concentration in bottles filled with filtrated surface water. The

bottles were suspended at 1 metre below the surface of water for 6 hours. Fig. 4 indicates the results of the experiment at Lake Nakanuma in July. Linear correlation between those two values, with limitation under 0.1 mg. chlorophyll/l, can be seen. This was also confirmed by Ryther (1956), though his experiment was done not in field, but in laboratory with cultivated algae. The highest chlorophyll concentration found in various Japanese lakes was in a range of 0.1 mg.-0.15 mg. per liter and the photosynthetic capacity (gross photosynthesis) of these waters was 8 mg.-14 mg. of O_2 per litre and day, namely the maximum photosynthesis in these waters could reach the values of 90 mg. O_2 /chl. mg./day. Manning and Juday (1941) reported that in water bloom the maximum photosynthesis per unit chlorophyll was usually not so high as expected, but under ordinary condition, the phytoplankton population is not so large as the density effect acts remarkably on the depression of photosynthetic capacity. The chlorophyll amount, therefore, can be used as a measure of productive capacity of a lake. It seems sometimes to be not proper to compare the productivity of lakes on the basis of chlorophyll concentration alone, because the photosynthetic activity of phytoplankton is determined by many factors. However, if the environmental factors, the productive structure of community, deterioration of phytoplankton, etc., are properly considered, the chlorophyll amount will be very useful as an index of photosynthetic capacity.

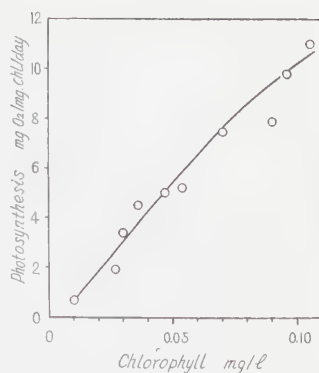


Fig. 4. Photosynthesis of phytoplankton in lake as a function chlorophyll concentration

E. Correlation between the productivity and the growth rate of phytoplankton population

In the early papers (1954a, 1954b), we analysed the seasonal change in standing crop of phytoplankton on the basis of the productivity, and it was confirmed that there was a parallelism between the productivity and the growth rate of phytoplankton. Such a parallelism has also been proved experimentally by Talling (1955).

The present study proved the results of these works with field experiments. The results obtained at

the depth of 1 metre in Lake Nakanuma in earlier part of 1956 are shown in Fig. 5. The feature of seasonal change in photosynthetic rate agreed fairly well with that

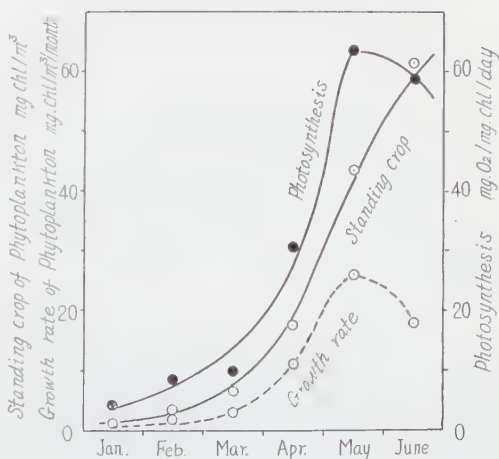


Fig. 5. Relation between productivity and growth rate of phytoplankton population.

of growth rate indicated by the increase of chlorophyll per month. As seen in the figure, however, there was a little discrepancy between variation curves of photosynthesis rate and standing crop during early summer. The photosynthetic rate and the growth rate were the highest in May and declined gradually thereafter, while the standing crop did not attain to the highest value at this time and increased until the end of July. Similar results have also been reported by Penfound (1956) who measured periodically the standing crop of *Typha latifolia*. To make clear the cause of the seasonal variation of phytoplankton, it seems to be necessary to analyse the proportionality between the photosynthetic rate and the growth rate on physiological basis in future.

Summary

- (1) Photosynthesis of natural phytoplankton was studied under field condition. The amount of phytoplankton was determined by chlorophyll content of lake water and the photosynthetic rate was expressed by oxygen in mg. evolved by one mg. chlorophyll.
- (2) The effect of diurnal change of light intensity on the photosynthesis was remarkable. The light saturation of the photosynthesis was found at about 50 per cent of the surface light and the further increase of light caused even inhibition in photosynthesis.
- (3) The photosynthetic rate of phytoplankton varied considerably in the course of a year and showed two marked pulses in early summer and in autumn. However, the feature of variation of photosynthesis much differs with lake types.
- (4) As the maximum photosynthetic activity under field condition, 80 mg.-90 mg. O_2 /chl.mg./day in eutrophic lake, 30 mg.-40 mg. O_2 /chl.mg./day in mesotrophic lake and 10 mg.-20 mg. O_2 /chl.mg./day in oligotrophic lake were measured respectively.
- (5) The photosynthetic capacity relates with the amount of chlorophyll fairly strictly.
- (6) Parallelism between the photosynthetic rate and the growth rate of phytoplankton population was recognized.

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維管束構成並びに走向に関する考察 トウモロコシの維管束解剖 第7報

熊 沢 正 夫*

Masao KUMAZAWA*: Considerations of the Vascular Construction with Special
Reference to the Vascular Course. Vascular Anatomy in Maize. VII.

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緒 言

筆者はトウモロコシの維管束解剖に関する第1報(1939)及び第2報(1940 a)で、雄性小穂及び雄花穂内の維管束走向を追跡し、第3報(1940 b)では稈の多数の節間に亘り葉跡条の行動を追跡した。第4報(1942)では、稈の節部に見られる網状に横走る維管束の発生・走向・他の垂直維管束との関係を調査したが、それに関連して稈中に存在して見られる個々の維管束の分化する順序等を知る必要に迫られて、その知見を第5報(1946)として公にしておいた。その後中断していたが、最近腋芽及び不定根と母軸との維管束連絡の所見を記述して第6報(1958)とした。これで既に Laubengayer (1946, 1948, 1949), Reeves (1946, 1950), Lenz (1948) などにより詳細に報告された雌花穂を除いては、一応トウモロコシ全植物体内の維管束の立体的配列について、自己の所見を得たことになった。尚筆者の第1報以後トウモロコシの形態・組織を取扱った報告が多数に出ているが、その内には筆者の既往の研究と重複したり、直接関係する点の多いものもある。従ってそれらの報告をも参照して、本報では主要な点について総合的考察を試みる。

前維管束の不連続分化

側枝や葉の如き側生器官の前維管束の分化方向については、母軸維管束から連続的・求頂的に進行する場合と、逆に側生器官内又はその基部附近に、先づ遊離して前維管束が出現し、これがその後求基的に分化して行き、遂に母軸維管束に接続する場合との2型式が報告されている。腋芽の前維管束分化が前型に属する例は Louis (1935),

Esau (1942), Reeve (1943), Sterling (1945), Kundu and Ras (1954, 1955), Sacher (1955), Philipson (1948), Lawalrée (1948), Garrison (1949, 1955) 等が報告するところで、Sterling 及び Sacher の所見は裸子植物についてであるが、他は双子葉類についての所見である。これに対し近代の著者としては Majumdar (1942), Wardlaw (1943), Majumdar and Datta (1946), Sterling (1947), Krauss (1948), Gifford (1951) 等が不連続分化の例を示している。これらの内、Krauss の示した *Ananas* のみが単子葉類に属する。

筆者のトウモロコシにおける所見(1958)によれば、稈の腋芽の場合では、初期の枝跡分化は不連続であり、前維管束は明かに求基的方向に分化が進んで、母軸維管束と接続するが、雄穂軸頂端部における小穂の場合には、その枝跡は求頂的に母軸維管束から伸びて行く。稈に着生する側生器官と花穂軸上の側生器官との著しい相違点は、前者が軸の成長点から相当距った部位において、母軸に較べて小型の原基として分化するに対して、後者は成長点に接近した部位において、既に母軸に較べて比較的大型の原基として分化することである。この事が枝跡の分化の求基的又は求頂的方向に深い関係があり、側生器官の形成と母軸の形成との間に、余り大きな時間的差異がない場合には、前維管束の求頂的分化が見られると考えられる。同様の結論を Wardlaw (1943) もシダの1種 *Onoclea* で与えている。求頂的分化と求基的分化とは、一応全く対立的な型式とも云えるが、上記の関係から考えると、恐らく組織分化の機構上の本質的差異によるものではないであろう。こ

* 名古屋大学教養部生物学教室 Biological Laboratory, Department of General Education, Nagoya University, Nagoya, Japan.

の事は葉跡条の前維管束の分化様式の所見からも云い得る。即ち Sharman (1942) によると、トウモロコシでは中肋以外の葉跡条の前維管束は、葉基附近から求基的に分化して茎中の維管束に連絡する。但し中肋となるべき葉跡の前維管束は節部附近 (disc of leaf insertion) に先づ独立に分化し、これが上に進んで葉に入ると共に、下方に進んで茎の既存の維管束と連絡すると、一応記述しているが確言を控えている。筆者の所見 (1946) では、最初に分化する中肋前維管束のみが茎中の維管束から求頂的連続分化をなして葉に入り、それ以外のものは求基的分化をなして母軸内の維管束に接続する。但し芽生植物の初期の葉にあっては、側肋前維管束も大型のものは中肋と同様求頂的分化をする。又 Esau (1953) によると、茎から葉への前維管束は多くの植物の場合、求頂的に分化し、原生節部も同様の方向に分化するに対して、それよりおくれて分化する原生木部は求基的に分化して茎の木部と接続する。Sharman (1942) のトウモロコシの場合には、原生節部原生木部共に茎から葉の方向へ分化するに対し、後生節部・後生木部の分化は求基的であるという。

尚前維管束の不連続分化はトウモロコシの場合、葉跡条と枝跡条に限るわけではない。即ち節間成長の著しい茎の部位にあっては、節部における方がその上下の節間部よりも維管束の成熟がおくれ、特に微細な最辺周部維管束の如きは、上下の節間で明白に観察できても、その間の節部附近では、それを前維管束の状態としても指摘できないことがある。これは節部附近は節間に較べて、その組織が永く分裂能を有しているため、組織の成熟がおくれ、特に小型の最辺周部維管束ではこれが目立つことによると考えられる。この点は既に第5報で述べておいた。ここで分化のおくれる部位を節部附近と云ったが、厳密には節間成長が初まった場合には節間分裂組織の存在する部位に相当するから、節間の下部と表現すべきであろう。然し実際は未だ細胞の分裂が節間分裂組織と呼び得る部位に限定されない時期のことであるから、このように呼んだわけである。

維管束系の二環構成

古くトウモロコシの維管束走向を研究したFalkenberg (1876), Strasburger (1891) 以来、茎

の中心部近くに位置する維管束は上位の側生器官から由来するもので、これを下方へ追跡すれば、結局最辺周部に存在する維管束の位置に立ち帰るものと信ぜられていた。

筆者 (第1報) は雄穂軸頂端の横断切片に現われている髓内維管束を、下方へミクロトーム横断切片により更に19節追跡して見た。19節とは同位小穂 (paired spikelets) 36個、第2次花穂軸7個及び葉6個が側生した主軸の区間を意味する。その結果最初の髓内条は相互間、或は後に側生器官から入って来た維管束と合体しつつ下降し、次第に茎の辺周部に移るが、最後の第49節を通過しても、尚最辺周部に位置する小維管束と同列にならず、それより稍内側に留まっていることを発見した。最頂端部で見られた髓内条に限らず、それより下位の側生器官から由来して髓内条となったものも全く同様の行動をする。従って主軸の最辺周部に位置している維管束は、決して髓内条の下端と連絡せず、独立の存在として、終始茎の最辺周部にあるので、この維管束を第2報 (p. 311) で最辺周部維管束 (Outermost peripheral bundle) と呼んだ。それに対し髓内条が相互の併合を繰り返して下降し、結局最辺周部維管束に接近して位置を占めても決してこれと合着しないものを第4報 (p. 530) で合成維管束 (Compound bundle) と呼んだ。維管束のこの2系統は節間の1横断面では判別が正確にはできない。両者が共に茎の辺周部にあり、かつその配列が或る程度重なり合うからである。然し節部の切片では、最辺周部維管束は明白な1輪をなし、他の髓内条と一見して判別できることを、第2報 (Fig. 3) 第3報 (Fig. 2) 第4報 (Fig. 3) で示した。但し合成維管束系の方は不規則な1輪をなすのみで、上位の側生器官から由来した維管束が、徐々に合成維管束列に参加するから、これと峻別することは不可能である。要するにこの2系統は相互に直接の連絡のない独立のもので、後に述べるように、すべての側生器官から由来する維管束は2系統にそれぞれ接続する。このような二環構造は小穂の柄の部分 (第2報) から初まり、下は第1葉節と幼芽鞘の着生する部位との間で2環が合体して1環となる。即ち茎の全長に亘る構造である。外環を構成する維管束は、外被穎の側肋から最初に由来するもので、その中肋から由来する維管束は内環に合着し、それ以高の水準では

二環構造は見られない。この点から云えば、外環維管束の方が内環のものより2 次的起原と見られよう。

このような二環構造の存在は第3 報(Fig. 1)以来、しばしば図示してこれを強調して来た。その後 Sharman (1942) はトウモロコシの稈において、維管束走向を多数の節間に亘り追跡したが、このような構造を認めず、*Laubengayer* (1946) の述もその目に触れなかったと思われる。然し更におくれて *Laubengayer* (1946) が人為的に軟弱な組織を解離させた雌穂軸について、初めて筆者同様維管束の二環構成に着目し、*Reeves* (1946, 1950), *Laubengayer* (1948, 1949), *Cutler and Cutler* (1948) も同様雌穂軸でこれを指摘するに至った。*Cutler and Cutler* だけは筆者の第1 乃至第3 報を一応引用文献中に掲げているが、実態に即した描写はなく、*Laubengayer* (1949) 他2 者の内 *Laubengayer* (1949) は雌穂軸以外に雄穂軸内の維管束走向を記述しているが、この部分は筆者の第1 報 (1939) 第2 報 (1940 a) と全く重複している。従って、雌穂・雄穂軸内の維管束の二環構成は全く同じであって、二環構造を予想外の所見と述べている。

筆者のいう最辺周部維管束・合成維管束の両群を *Laubengayer* (1948) はそれぞれ Outer system, Inner system と呼び、*Reeves* (1950) は Peripheral system, Axial system と呼んだ。雌穂軸内で二環構造を指摘した *Reeves* (1953) も稈の内にはこの構造の存在を今尚気づくに至っていない。

節 網 維 管 束

葉節部(雌穂軸に苞の着生する節も含む)に見られる複雑な網状の横走維管束、即ち筆者の節網維管束と呼ぶものの起原・本性・意義については、従来種々の見解があるが、第1 報でその起原と構造について詳細に報告しておいた。

節網維管束は *Arber* (1930) の云う如く基本組織の再分裂に由来するものではなく、茎の多くの髄内条が前維管束として分化した後に、爾余の基本分裂組織中に分化するもので、垂直維管束に較べて二次的である。然し垂直維管束の分化は第5 報で示したように、茎の中央部から最辺部へ進むので、茎の最辺周部維管束及び合成維管束が前維管束として分化する時期と、その附近の基本分

裂組織から節網維管束が分化する時期との時間的隔りが少い。従って節網維管束はそれ等の維管束と最も密に結合し、髄中へ外から入りこんだばかりの葉跡条とは、原則として全く連絡がないことは、節網維管束とその葉跡条の分化時期の隔りからも諒解される。

節網維管束と他維管束又は他器官との連絡関係については、*De Bary* (1877), *van Tieghem* (1884), *Guillaud* (1878), *Strasburger* (1891), *Bugnon* (1920), *Arber* (1930) その他の見解があるが、比較的近年の *Sharman* (1942) のトウモロコシの稈によれば、節網維管束は葉よりも不定根の維管束と連絡するとなし、又辺周部維管束の直接の連絡とも述べている。然し彼の研究は、*Laubengayer* (1946) の著目したものではないから、記述も詳細に亘っていない。

筆者のその後の研究(第6 報)によると、母軸中に入った枝跡条の下端が節網維管束状に横走してその行方を見失うこと、或は又茎と不定根とを連絡する維管束が同時に茎の中心部の方向へ節網維管束状に横走する所見が得られている。然し枝跡・葉跡・根跡が節網維管束に起原を有するとなし得る確実な所見は殆ど得られていない。又側枝や不定根を具えない節でも、大型の葉器を具える節には、必ず節網維管束が発達する事実からすれば、側枝や不定根の維管束と節網維管束との連絡が実際に見られる場合でも、その連絡は基本分裂組織中における両系統維管束の分化時期の接近している場合に起きる偶発的のものと考えられる。又一方大型の葉器を伴う節に例外なく節網維管束が発達する点から云えば、たとえその節において髄に進入して来た葉跡条と直接の結合がなくとも、何等か未知の生理的関連が両者の間に存在するものであろう。

葉と茎との維管束連絡

トウモロコシの葉跡条の走向は古い時代からしばしば研究されたが、尚不十分なので、筆者は主軸上に着生する葉数(平均 11 葉)の少ない早生品種赤四十口を選び、稈の8 節間に亘り葉跡条の走向を追跡した結果を第3 報に報告し、その中で葉跡条走向の諸型を模式図(Fig. 1)で示した。筆者は葉跡条をその走向の上で次の3 型に便宜的に区分しておいた。

第1 型: 最辺周部維管束環を通過して多少に

拘らず随に入り込むもので、大型のものは最も深く、小型のものは浅く入り込み、その間に種々の程度があって、Strasburger (1891) はその程度により 3 群に細分した。

第 2 型：その節において随に入りこむことなく、直に最辺周部維管束に合着する小型の葉跡条で、Strasburger はこれを更に 2 群に区分している。

第 3 型：茎の皮層に入り最辺周部維管束列にまで達せずして消滅するもので、葉の裏面近くに位置し、機械組織で包まれた微小な葉跡条である。

第 1 型の葉跡条は随に入るまでは、節部の外側に多量の機械組織を伴うが、随に入り込む前にこの組織は消滅するか、又はこの組織だけ葉跡から分離して皮層内に留まり間もなく消失する。然し发育のよい個体にあつては、その機械組織中に 1~3 個の微小な維管束を含んでいる。これを Esau (1943) は独立の維管束が共通の機械組織で包囲されたものと見ている。筆者 (第 2 報) が走向を追究した結果によると、この微小維管束は葉の中では見られず、単に大型葉跡条が母軸節部の皮層内を貫く間だけ姿を現わすもので、間もなく大型葉跡条と分離して、最辺周部維管束に合着してう。従つてやはり第 2 型の葉跡条の 1 種と見なしておく。

走向が問題になるのは第 1 型の葉跡条についてである。筆者が得た所見は第 1 節で随の深部に入り込み、第 2 節以下で順次辺周部へ移行するもの、第 1 節では比較的浅く入り込み、それ以下の 1-2 節で更に深部へ入り、その後再び辺周部へ逆行するものがある。従来の報告と異なる主要点は次の通りである。

- 1) 随へ入り込んだ葉跡条が辺周部へ移行するとも、決して最辺周部維管束環へは入らず、それより稍内方に留まっていた、随内性を呈の最下部まで失わない。換言すればそれは最後には筆者のいう合成維管束系を構成するわけである。
- 2) 葉跡条は下方の節で合成維管束環に合着する以前にも、同一葉から由来した葉跡条は他の葉から由来した葉跡条としばしば合着し、これが随の深い位置でも起きる。
- 3) 随へ入つて来た葉跡条は下位の節部で辺周部へ移行するに当り、必ずしも最初に随へ入つて来た方向の辺周部へ逆行するとは限らず、甚だしい場合には正反対の方向の辺周部へも移行する。

尚葉跡条が他の葉跡条と何節間併合されずに随走するかは個々によって異なり、小型のものは 1 節間随走して合成維管束環に入り、中肋に由来する葉跡条は 7 節間以上も独立に随走するものもあるが、側脈から由来するものはもっと早く他葉跡条と合併された後、合成維管束環に入るのを通則とする。

筆者の報告後 Sharman (1942), Esau (1943) がやはりトウモロコシの葉跡条を多くの節間に亘り追跡したが、筆者の所見に追加したり、これと一致しない部分は二環構造の点を除いては何もない。即ち筆者は葉跡条の下端は決して最辺周部維管束環に到着せず、この意味で典型的な Y 型走向でないとなすに対して、両名は最辺周部維管束群が随内葉跡条に關係のない独立の存在であることに気づいて居らず、葉跡条は結局茎の辺周部へ移行して他維管束と併合されるとなすに留まっている。両名以後にはトウモロコシの葉跡条の行動に関する報告はない

側枝と主軸との維管束連絡

側枝母軸間の維管束連絡についての総合的所見は Miller and Wetmore (1946) も指摘する通り、現在未だ不十分であるが、特にイネ科にあつては多数の散在維管束と節網維管束の存在により、正確に追究するのは困難である。従つて古く de Bary (1877) 以来 Bugnon (1920, 1924), Arber (1930) その他の研究もあるが、甚だ概略的な所見しか得られていない。たとえば de Bary の記すトウモロコシの場合では、枝跡は節で不規則に横走し母軸の辺周部維管束と合着するとあるだけで、基本的な維管束連絡の様式を理解することが出来ない。ミクロトーム切片による Arber の研究は、側芽中の少数の維管束を母軸の方へ追跡したもので、その走向を正確に捕えたとしても、側枝・母軸間の維管束連絡の全貌を示すものではない。

トウモロコシの場合、側枝として最も維管束配列の単純なのは雄穂軸頂端附近に着生する小穂である。小穂は普通 1 対が左右に並び基部が合一し、母軸の 1 個所に着生しているので、筆者 (第 1 報) はこの 1 対を同位小穂と呼んだ。これは軸上の 2 個所に生ずべき 2 個の小穂が癒合したものとも考え得るが、本来は 1 個の蓋葉で覆われた 1 個の側枝であることは Lindstrom (1925), Wea-

therwax (1927), Singleton (1947), Cutler and Cutler (1948), Reeves (1950), Nickerson (1954), Galinot (1954) などの所見からも疑ない。

同位小穂と母軸との維管束連絡は既に第1報・第2報で述べたが、その結果として得られたものが第6報 Fig. 2, 3~4 である。即ち各小穂基部では中央に数個の維管束が密集して1塊をなし、その外側に数個の小維管束が皮層条の觀を呈して存在するが、同位小穂の中央維管束は母軸への着生点で一時全く合体して、第2報 Fig. 1, 4 に見る如く、並生維管束が環状に配列した1群をなすが、直にこれが左右に2分して母軸の合成維管束と合着する。双子葉類の葉脈の形成は、母軸から左右から1個ずつ派出し、これが合体して背腹性な側枝の維管束となるを普通の型式(Esau (1953) Eames and MacDaniels (1947)) とするから、この型に照らせば、トウモロコシの葉脈の形成は違くない。但し維管束が双子葉類と異り連続した環状をなさないから、母軸に明白な枝隙を指摘できない。Reeves (1950) によると、小形の雌穂軸では同位小穂への枝跡として単に1個の維管束が母軸維管束から分離して来るとなしているが、筆者の見た雄穂軸の場合には、最も簡単な場合でも母軸から2個の枝跡条が派出し、互に合成維管束(第6報 Fig. 2, 3) について見れば、母軸に面しない方向の側枝最辺周部維管束はそのまま母軸に入り、他のものは母軸の左右の最辺周部維管束に合着する。この枝跡条の派生から、互に連続したとすれば、円弧状の1維管束が形成されるわけで、その中央に葉隙が存在すると假定すれば、双子葉類の単隙型(Unilacunar type)の枝跡の行動と同一様式に還元できる。唯ここに注意すべき点は同位小穂基部にあっては、向軸側に小維管束が存在しないこと及び母軸の側枝側には辺周部小維管束の分化が悪く、しばしば下部が盲管として消滅(第6報 Fig. 2, 3) することである。然しこれが簡略化の1表現に過ぎないことは、以下記述するところから明かである。

第2次雄穂軸とその母軸間* 及び同位小穂とその母軸間との維管束連絡(第1報 Fig. 3)を比較するに、前者の場合では1) 母軸・側枝共に維管束の数が多く、2) 最辺周部維管束と合成維管

束とがその配列の上で相当乱れていること、3) 母軸の側枝側最辺周部維管束が良く発達すると共に側枝の母軸側最辺周部維管束が存在すること、4) 側枝の合成維管束の或るものは母軸中に入り、他の維管束と直接合着せず、そのまま母軸中を下降する点で後者の場合と異なる。しかし側枝の最辺周部維管束は母軸のそれに、又側枝の合成維管束は母軸のそれに合着する点では、両者の場合全く同一である。後者の場合、同位小穂の中央維管束をなす枝跡が母軸の2個の維管束から由来するに反し、前者の場合母軸の数個の合成維管束から枝跡が分岐して来ることは、維管束の数の増加に伴う必然の複雑化の結果に他ならない。

第6報で初めて記述した蓋葉に伴われた側枝と主稈との維管束連絡は、上記の場合より更に格段に簡略化しているが、これは実は花序軸には見られない節網維管束と蓋葉の葉脈条との存在及び維管束の数の著しい増大に附随する結果である。節網維管束は第4報で示した如く、組織発生的には他の維管束よりも後発的起源のものであるから、これを一考慮外におくと、この場合の維管束連絡の様式は、最も簡単な同位小穂とその母軸との維管束連絡を原型として容易に諒解し得るところである。同時に又双子葉類の普通の枝跡条の母軸に対する関係との相同をも察知することができる。唯双子葉類の多くの場合と著しく異るところは、トウモロコシの場合茎軸内の維管束が二環構成であり、枝跡条がこの二環に対し同様の連絡関係を示すことである。

不定根と茎との維管束連絡

不定根と母軸との間の維管束連絡を見るに、de Bary (1877) によれば、禾本では根跡が茎の中央まで入り込み、その附近で垂直維管束と合着するとなしているが、筆者のトウモロコシにおける研究から推せば、彼の記述は節網維管束の走向と本来の根跡条の走向とを混同した所見であると信ぜられる。Sharman (1942) は軟弱な組織を解離させた材料について、節網維管束が不定根と固連することを記述しているが、これは充分成熟した程の基部における材料であり、恐らく解離に堪えた多量の機械組織の存在によって、根跡条の

* Laubengayer (1949) は連続切片の図を提示しているが、詳細な記述や模式図を伴っていないので、何等これから実況を知ることができない。

真実の行動を諒解する上に若干の疑問がある。

Bugnon (1920, 1924) の禾本, Krauss (1948, 1949) の *Ananas*, Mann (1952) の *Allium* についての所見その他では、茎の最辺周部の維管束から根跡が発端する。第6報で示した筆者のトウモロコシの場合でも、不定根の発端部位は同様であり、根跡は根の発端部位に近い茎の最辺周部維管束の数個と結合すると共に、尚その上に附近に存在する合成維管束とも連絡することに注意しなければならない。根跡が直接茎の髓を横走して反対側の辺周部維管束環にまで発達するような所見は得られなかった。但し根と茎との合成維管束を相互に連絡する横走維管束が、 Γ に若干深部に入り込んで節網維管束状をなすことは見られたが、筆者の材料ではこれが髓の中心部まで分布する例はなかった。又筆者の所見では、この節網維管束は葉跡が茎に入り込む部位に良く発達している節網維管束とは明らかに上下に分離している。しかし節間の短縮している稈の基部では、両者が相接触して良く発達するから、この場合には根跡条が茎の中央部を横断する靱を与えることもあり得ると考えられる。

以上によって、側枝・葉及び不定根の維管束はすべて等しく茎の内外二環の維管束系、即ち最辺周部維管束系及び合成維管束系に結合することが明かとなった。この点に関する限りこれらの側生器官相互の間には全く差異がない。尚葉性側生器官と茎軸性側生器官とを比較するに、これらの側生器官から由来する個々の維管束の下方への走向や他維管束との結合状況にも本質的な差異は見られない。唯普通の葉器は母軸の全周に亘り葉跡を送り込む多隙型 (Multilacunar type) であり、花部を構成する小型の葉器 (内被類以上のもの) の場合のみ単隙型であるに反し、茎軸性側生器官にあっては、大型の葉腋側枝たると小穂の如き小型のものであるとを問わず、常に母軸の1局部に枝跡を送り込むので、単隙型と見做し得る点にある。枝跡の単隙型は少数の例外を除いては、高等植物の通則であり、この点でトウモロコシも双子葉類の他と規を同じくするといえる。又茎軸性器官は葉性器官と異なり、本来は背腹構造を示さないことが両器官の重要な形態学的特性であるが、枝跡の行動その他の上でもやはり背腹性の徴候はない。強いていえば、側枝の向軸側最辺周部維管束の分化発達が背軸側のそれに較べて若干劣

る程度に過ぎない。

上記2種の側生器官は母軸内維管束の一部を多少に拘らず自己の内に取り込み、母軸維管束の走向・配列に影響を及ぼす点で相類似する。それに反し、不定根が茎から出る場合には、母軸維管束の走向・配列に対して何等の影響を与えない。この点から見れば、不定根は他の側生器官即ち葉及び側枝の場合より、母軸との間の解剖学的関連の稀薄な器官ということができよう。

単子葉類維管束構成の二環説

トウモロコシの茎軸内には、終始相互に直接の連絡のない2系統の維管束が二環をなして内外に重複して居り、すべての側生器官の維管束はその2系統に同じ型式で連結していることが、筆者の所見により始めて明白になった。

これに類似して二環構造を示すと思われる単子葉類の古くから知られた例として Falkenberg (1876) の *Hedychium*, Guillaud (1878) の *Tradescantia* や *Acorus*, Gravis (1898) の *Tradescantia* などがある。*Acorus* の根茎における2系統の維管束は茎の比較的辺周部に位置し、小型葉跡条は直に外環をなす維管束又は内環をなす維管束に合体するに反し、大型葉跡条は一度茎の髓深くへ進入してから改めて辺周方向へ立ちもどり内環を構成する維管束に合体し、この点トウモロコシと同様である。*Hedychium* や *Tradescantia* の場合には内環を構成する維管束は茎軸維管束と呼ばれ終始茎の中央近くに位置し、大型葉跡条は之に合体する。de Bary (1877), Gravis (1898) によれば、*Tradescantia* では根跡条も内外2系統の維管束に等しく連絡し、この点もトウモロコシと同様である。Guillaud (1878) や Plowman (1906) の *Scirpus*, *Dulichium*, *Luzula* 等の場合、やはり二環構造であるが、内環は茎軸維管束として取扱われ、葉跡は内環と関連なく、枝跡はこれに反し外環に関連なく内環に併合されるという。若しこれが真実であるとすれば、内外二系統の維管束に対する側生器官の関係がトウモロコシやツクサ科の場合と異なるわけであり、この点今後の検討を要する。

筆者は多数の散在維管束を有する単子葉類の種々の科の代表者について、多くの節間に亘り葉跡走向を追跡して見た。その結果多くの種属において、トウモロコシやツクサ科と同様、二環構成

を基本型式と見做すことができ、これが単子葉類の1代表型式であるが、唯多数の維管束が散在しているため、一見甚だ複雑になっているにすぎないと思われるに至った。この見解を奥村 (1950) 仮りに単子葉類維管束構成の二環説と称しておいた。尚過去 100 年来単子葉類葉跡条の典型的なヤシ型走向——葉跡条が髓走した後、その最下端では茎の最辺周部維管束列に加わる如き走向——として知られて来たような葉跡条とは異なるものとしても、極めて稀なものであることを前 (1949)

に指摘しておいた。

既往の研究者によって一応記載された種属にあつても、維管束が多数であり、その走向の複雑なものは更に詳細に検討される必要がある。

若干の引用文献入手につき、当時滞米中の木村資生博士の助力を得たので、ここに謝意を表する。

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Summary

In this series of papers, the writer has traced the vascular strands of the spikelet (1940a), the male inflorescence axis (1939), the axillary shoot (1958), the leaf (1940b) and the adventitious root (1958) to the mother axis with a view to examine their behaviours, and he has also studied the origin and structure of the nodal plexus (1942) and the developmental order of the vertical bundles of the stem (1946). Consequently general consideration to the vascular construction has been given in this paper with special reference to the vascular connection of the lateral organs with their mother axis.

As pointed out previously (1940b, 1942), two systems of vascular bundles, one inside of the other and independent without being directly connected with each other, are distinguished throughout the cauline axis: the outermost peripheral bundles and the compound ones constitute respectively an outer system and an inner system at rather peripheral regions of the axis. The trace bundles from the lateral organs, i. e. the spikelet, the secondary inflorescence axis, the axillary shoot, the leaf and the adventitious root, are equally connected with those two bundle systems of the mother axis. Although in the case of the lateral shoot subtended by a foliar leaf, the behaviour of branch trace strands are greatly modified and complicated, owing to a large number of bundles, the presence of leaf traces and the development of nodal plexus, the fundamental pattern of the vascular connection with the mother axis is interpreted to be homologous to the case of dicotyledonous plants, if the horizontal vascular plexus is left out of consideration. The only significant difference of vascular construction between maize and dicotyledonous plants is that the vascular system in maize is of dicyclic construction from the first foliar node upwards to the pedicel of the spikelet.

The lateral organs of phyllome nature, except the floral phyllomes, bring about the multilacunar nodal structure of the mother axis, while those of cauline nature bring about the unilacunar, regardless of their vascular constructions which are complicated as in the axillary shoot or simple as in the pedicel of the spikelet.

The writer has studied the vascular courses in some representatives of monocotyledonous families. Although the results of his special observations have not yet been published, he has formed a conception that the scattered vascular system of many monocotyledonous plants may be interpreted as of the dicyclic construction, the inner vascular system being situated at the centre of the axis in some species and at rather peripheral regions of the axis in other species. The dicyclic theory of vascular construction in monocotyledons was advanced by the writer (1950) preliminarily in Japanese.

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SHORT COMMUNICATION

Flower Promoting Effect of Gibberellin
in *Erigeron annuus*

by Mitsuro OKUDA*

奥田七郎*: ヒマジョウソウに対するジベレリンの開花促進作用

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Erigeron annuus, a biennial plant, remains in the rosette form in the first year under either short, long, or natural day condition, but flowers readily under long day condition when it is vernalized by cold temperature. Plants in rosette form were collected and transplanted in 20 cm pots, and they were grown under short or long day condition. Aqueous gibberellin** solution of 10 mg./l was sprayed on leaves every day for a month from June 8, 1957.

Consequently, bolting and flowering were observed after 60 days from the beginning of the treatment under long day condition. Under short day condition bolting was observed but no floral initiation was produced. Stem elongation ceased if the gibberellin treatment was discontinued and the plants formed rosette leaves again. In such plants, flowering was observed without stem elongation, if they were transferred to long day condition.

Various kinds of abnormality were observed in the plants treated with gibberellin, they were as follows; 1) colour paler than normal, 2) abnormality in the leaf shade, 3) slender stem, and 4) poor hair on the stem.

From the above mentioned results it was deduced that gibberellin has the same effects as "Vernalin" for *Erigeron* plant.

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* Biological Institute, Yoshida College, Kyoto University, Kyoto. Japan.

京都大学教養部生物学教室

** The used gibberellin is the product of Kyowa Fermentation Industry Co. Ltd., Tokyo, and is the mixture of gibberellin A₁ and A₃. Gibberellin A₃ is gibberellic acid and is contained more than 80 per cent of the mixture.

略 伝

自然を愛した人・教師・研究者・松浦茂壽氏の一生

早くからの本会会員松浦茂壽氏が亡くなられた。

(昭和32年9月20日、享年59才) 氏は高知県の人、高知師範在学中から生物特に植物学に心を惹かれ、同校卒業後同県の尋常小学校に勤務の数年間にも斯の道への志益々やみがたく、遂に東上、当時駒場にあった東京帝国大学農学部附属教員養成所に入学、家事の都合によってことを中途退学したところまでが同氏が学校で受けた教育である。

その後高等学校生物科助手、その間に教員検定試験を通り、昭和2年当時の小田原中学に教諭となられて以後の33年間は小田原中、小田原高の松浦先生として極めて熱心、又かなり高名な博物教師として通って来られた。但しこの方面における貢献については他の機会にゆずり、ここではもっぱら氏の植物学について述べることにする。

氏の研究は極めて一般的な意味での博物学探求にはじまる。すなわち殆ど当然のこととして山に海に或は植物の或は動物の採集会の定連であり、常に野山に生徒を連れ出す博物の先生であった。これを機縁として多くの植物学者、動物学者との交遊がはじまり、常に多くの生物好きの生徒たちを身近にあつめた。またしばしば単身採集旅行をくわだて、その足跡は南樺太から琉球に及び、特に興味をひいた地方には、年中足しげく通いつめ、なめるように調べ上げるのを得意とした。一地方の博物誌を編むことである。実力とはこうして育成され、磨かれたものの興味とこの方面の活動はもっぱら居住する箱根、小田原地方への愛情と表裏し、その結晶したものが氏の「相模湾海藻誌」、「箱根植物誌」である。この方向の活動と熱心は、自然に氏をこの地方の博物



ス(?)にした。昭和24年創立された小田原生物同好会、昭和31年発足した箱根博物会はいずれも氏をその生みの親、育ての親とするものである。最近における氏の「一生の念願」は箱根博物館の設立であった。この夢が氏の生前、現実となるに至らなかったのは残念であるが多くの後援者の協力によって近い将来には実現され氏の霊をなぐさめるであろう。

他方、氏の『研究がしたい』と云う念願は上記の長い博物学彷徨の間に、そして多忙な教師としての生活の間に、次第にその焦点を海藻学に見出したようである。上記の博物誌的活動にくらべてこの道は必ずしも近づきすやくはなかった。然し、氏の歩みは実にゆっくりと、しかしねばりづよいものであってはじめは岡村金太郎氏に、のちには山田幸夫氏に師事し、本格的な海藻学の勉強をコツコツと積み重ねること何十年、そして、次第次第にそれをカタチある研究にしぼって行ったものが相模湾海藻の分布、発生の研究であり、その最後になってしまった本誌所載の論文「真鶴岬における海藻発生の年周的観察」(本号93頁所載)である。氏の熱心と精進ぶりとをここにくわしく述べる紙面を持たないのは心残りである。

ここには氏が数年の間その精魂をそそいだ真鶴崎の岩礁上にある遺影をしかけるにとどめる。(上記論文中、本号101頁参照)

氏を知るすべての者の言うように、まことに温い人であった。時に奇行も少からず、本当に面白い人柄であった。然し、ここに筆者がこの学術誌の紙面をかりて氏を悼む一文を捧げる理由は、それら個人的な懷顧愛惜の情いかに氏がこの国の(又氏自身の)あらゆる困難と制約をも含めた現実の中にあつて常に自然を愛し、生物を愛し、美しい夢を持ちつづけて行かれたその姿の中に、真実生物を愛する生物学者の生涯の一つの典型を見ると信ずるからである。

東大理学部植物学教室 高宮 篤

Histochemical Studies on the Starch Formation from Various Sugars in Relation to the Permeability of Potato Tuber Cells

by Shichiro HORI*

堀 七 郎 : 貯蔵器官の形成に對する糖々の透かしのいふいふ一試とその細胞の透過性との關係についての組織化学的研究

Received November 22, 1957

There is no doubt to consider that the penetration of sugars into the cells of storage organs is an essential step for the starch accumulation in these organs. Nevertheless, plant cells are generally known to be slightly permeable to sugars (Yamaha, 1949; Curtis and Clark, 1950). Previous findings of the author (Hori, 1954, 1955, 1956) that a considerably long time was required for the formation of starch from sugars within the cells of storage organs such as potato tuber, maize kernel and sweet potato tuber seem to indicate low permeability to sugars of these organs. In our studies it was observed that from certain kinds of sugars starch was formed abundantly without plasmolysis of the cell, while none or but slight amount of starch was produced from other sugars which caused in most cases plasmolysis of the cell. These findings suggest the existence of a trend that sugars favouring the starch formation in the cell are more permeable and *vice versa*.

In view of these observations the author tried to investigate the starch formation in potato tuber cells from various sugars in connection with the permeability of the cells to these sugars.

Materials and Methods

Materials used in this investigation were small-sized, starch free potato tubers, less than 4 mm in diameter and almost at the developmental stage of so-called "stolon". The method of removing storage starch from the tubers was the same as described previously (Hori, 1954, 1956).

Sugars used in the experiments were sucrose, maltose, lactose, D-glucose, D-fructose, D-galactose, D-mannose, D-xylose and L-arabinose. Concentrations of the sugar solutions employed were 0.2, 0.5 and 0.8 M.

About 2.0 ml. of each sugar solution was put in a small Petri dish of about 45 mm in diameter, in which 3 or 4 starch free tubers were inversely placed with the stalks immersed in the liquid, so that the tubers were exposed to air. These Petri dishes with the tubers were then placed in another large covered Petri dish containing a

* Biological Institute of Kushiro Branch, Hokkaido Gakugei University, Kushiro, Japan. 北海道学芸大学釧路分校生物学研究室

small amount of water at the bottom to prevent the tubers from drying as well as to prevent the change of sugar concentration by evaporation and kept in an incubator at constant temperatures ranging from 15° to 30° for various periods from 4 to 48 hours.

After periods of 4, 24 and 48 hours, microscopic sections were prepared from each tuber and inspected for the occurrence of plasmolysis as well as for the formation of starch with iodine. As a control tubers were similarly incubated in tap water in place of sugar solutions.

Results

Tubers were incubated respectively in 0.2, 0.5 and 0.8 M solutions of sugars at different temperatures from 15° to 30°. The results are given in Table 1.

Table 1. Starch formations and occurrences of plasmolysis in potato tuber cells incubated in various kinds of sugar solutions as well as in tap water at different temperatures.

Temperature(C°)	15°			20°			30°		
Concentration(M)	0.2	0.5	0.8	0.2	0.5	0.8	0.2	0.5	0.8
Incubation time(hr.)	4 24 48	4 24 48	4 24 48	4 24 48	4 24 48	4 24 48	4 24 48	4 24 48	4 24 48
Sugars tested									
Sucrose	++	++	⊙⊙	++	++*	⊙⊙	+++*	++*	⊙⊙⊙
Maltose	++	○	⊙⊙	++	++	○	+++*	++	⊙⊙⊙
Lactose	++	○	⊙⊙	++	○	⊙	++	⊙	⊙
D-glucose	++	++	⊙⊙	++	++	++*	++*	+++*	++
D-fructose	++	○	⊙⊙	++	++*	○	++*	○	⊙⊙⊙
D-galactose	++	○	⊙⊙	++*	○	⊙⊙	+	○	⊙⊙
D mannose		○	⊙⊙		○	⊙⊙	●	○	⊙⊙⊙
D xylose	++	○	⊙	+	○	⊙⊙	+	○	⊙⊙
L-arabinose		○	⊙⊙	●	○	⊙⊙	●	○	⊙⊙
Control in water				48 hr. ●			48 hr. ●		

Starch formations are indicated in: +, starch size of 1—2 μ ; ++, that of 3—4 μ ; ++, that of 5—6 μ ; and +++, that of above 7 μ . The mark * represents no more formation of starch after further 23 hours. Occurrences of plasmolysis are indicated in: ○, occurred slightly; ⊙, occurred less strongly; and ⊙, occurred strongly. The sign ● indicates an occurrence of decay.

It can be seen from this table that at a concentration of 0.2 M none of the sugars employed caused plasmolysis. But, in the experiments with higher concentrations of the sugars applied, more or less strong plasmolysis occurred in all of the tuber cells

Those sugars which caused the rot of the tubers during the incubation seemed to be rather harmful to the tuber cells under these conditions of incubation.

Of the sugars used, D-mannose and L-arabinose failed to form starch at all at every temperature tested. From other sugars starch was shown to be formed. However, the amount of starch produced in the tubers varied considerably depending on the kind of sugars and temperature. It was observed that the increase in concentration of sugars resulted in the diminution of the sugar kinds capable of forming starch in the tubers with the concomitant increase in frequency of the occurrence of plasmolysis at every temperature tested. Glucose was the only sugar capable of forming starch with a high concentration of the sugar, but the amount of starch produced remained small. It seems interesting that the starch formation always occurred only in the tuber cells which showed no plasmolysis.

The amounts of starch indicated in the table were determined roughly by the estimation of size of starch grains in the same way as reported previously (Hori, 1956).

It was thus found from these results that the starch formation from sugars in potato tuber cells was markedly influenced by the concentration of sugar applied and the temperature at which the tuber was incubated. From this point of view, the present data do not contradict with the results obtained previously by the author (1954) that 10 and 1.0 per cent (=ca. 0.55 and 0.05 M) solutions of fructose, and 10 per cent solution of galactose did not cause starch formation in the tuber cells at 25°–30°.

Throughout the experiments, the tubers in which cells were plasmolysed became more or less soft, and no deplasmolysis could be observed within 48 hours. However, upon transferring the plasmolysed tubers back to water, the plasmolysis disappeared indicating that the cells were not irreversibly injured.

Starch free tubers treated with tap water in place of sugar solutions formed no starch at all and tended to rot later. The decay took place rapidly at higher temperatures, viz. within 48 hours at 20°–30°, while at a lower temperature of 15° the tubers survived more than 48 hours. On the contrary those tubers incubated in sugar solutions, and forming starch in the cell became more healthy than ever, with their tissues turgid and opaque in colour.

Discussion

Of the sugars tested for the starch formation in the potato tuber, D-mannose and L-arabinose were found to be completely inert. As these two sugars did not form starch even at a concentration of 0.2 M, where no plasmolysis was observed, the inability in starch formation of these sugars cannot be ascribed to any injury which should be indicated by plasmolysis of the plasma-membrane on account of their possible impermeability. It may well be that the tuber cells are lacking in the enzyme system which converts these two sugars into starch.

A noticeable parallelism was found between the presence of plasmolysis and the absence of starch formation. It is considered probable that the tuber cells are able

to take up sugar molecules from hypertonic solution, in which plasmolysis naturally takes place. The lack of starch formation, as the author has found, by tuber cells in such solutions indicates that some changes in the physico-chemical properties of the plasma-membrane can occur as a result of plasmolysis, leading to the blockade of the sugar penetration.

The degree of plasmolysis which occurred in the tuber cells at comparable sugar concentrations varied depending upon the type of sugar with which the tubers were incubated. From the degree of plasmolysis, the rate of penetration of the sugars may be roughly estimated as follows:

Glucose>sucrose>maltose and lactose>fructose, galactose and mannose>xylose and arabinose.

Obviously these results cannot be explained by a simple mechanical model presumed in terms of the "Ultrafilter theory" of Traube.

On the other hand, availability for starch formation of sugars can be obtained from the amount of starch formed from these sugars. Approximately, it is in the following order:

Glucose and sucrose>maltose>lactose>fructose and galactose>xylose.

Between these two sugar series a marked parallelism can readily be noticed. These results seem to indicate that those sugars which induce more starch formation are more permeable to the cells of the potato tuber.

Summary

The starch formation in potato tuber cells from various sugars was investigated histochemically with reference to the permeability of the cells to these sugars.

The availability of sugars for the starch formation in potato tuber was as follows:

Glucose and sucrose>maltose>lactose>fructose and galactose>xylose.

Mannose and arabinose were ineffective for the starch formation.

From the plasmolysis data the permeability of the sugars tested to the tuber cells may be considered to be in the following order:

Glucose>sucrose>maltose>lactose>fructose, galactose and mannose>xylose and arabinose.

The author wishes to express his sincere appreciation to Prof. T. Miwa of the Tokyo University of Education for the preparation of this manuscript.

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Studies on the Japanese Hyphomycetes (IV) Miscellaneous Group

by Keisuke TUBAKI*

椿 啓介*: 日本産不完全菌類の研究 (IV) 追加菌

Received January 14, 1958.

In continuation to the preceding papers in which the author enumerated the members of Hyphomycetes on such substrata as dung (1954), higher fungi (1955) and fresh water (1957), he proposes now to enumerate some fungi which are observed on various natural sources. In this fourth report, some additional and miscellaneous species of Hyphomycetes are described together with a species of the Zoopagaceae which is treated as an imperfect fungus in this study.

Acaulopage tetraceros

Drechsler, in *Mycologia* **27**: 195 (1935); Duddington, in *Trans. Brit. Mycol. Soc.* **33**: 121 (1950).

Mycelium slender, non septate, sparingly branched, 1-2 μ in diam., hyaline. Conidia borne singly on the apices of short branches of the hyphae, inverted bottle-shape with usually three to five, needle-shape, empty appendages which are produced from the broad distal ends of conidia. Conidia, 18-25 (27) \times 5.5-9.0 μ , hyaline; appendages, 15-25 \times 1.0-2.0 μ , hyaline.

Hab. On submerged leaves of *Cercidiphyllum japonicum*, Bot. Gard., Sapporo, Hokkaido (July, 1956).

This species was described by Drechsler as predacious on Amoebae and other Protozoa, capturing their prey by adhesion

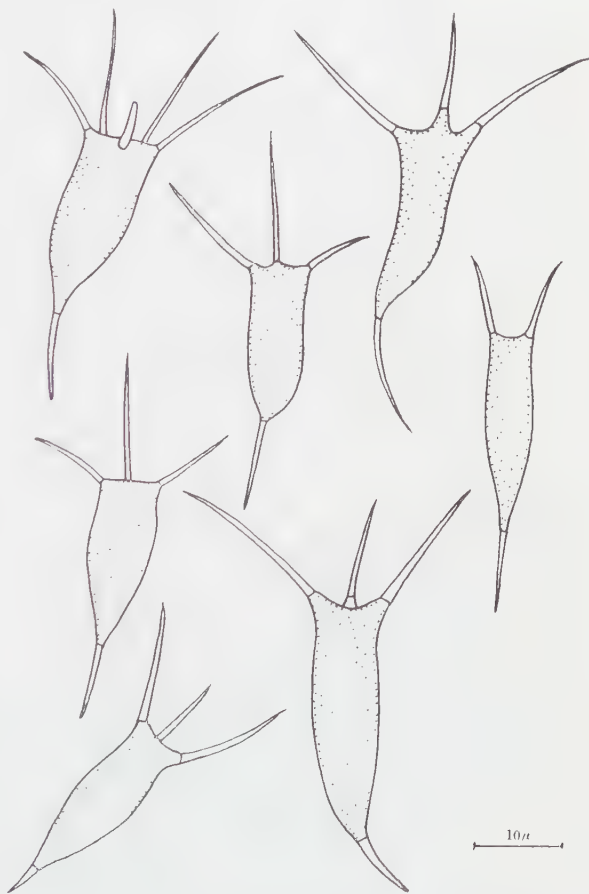


Fig. 1. Aquatic spores of *Acaulopage tetraceros*

* Nagao Institute, Kitashinagawa, Tokyo, Japan. 長尾研究所

to the mycelium. However, this capturing organ could not be observed in the present strain at all.

This strain could not be cultivated on any usual culture media.

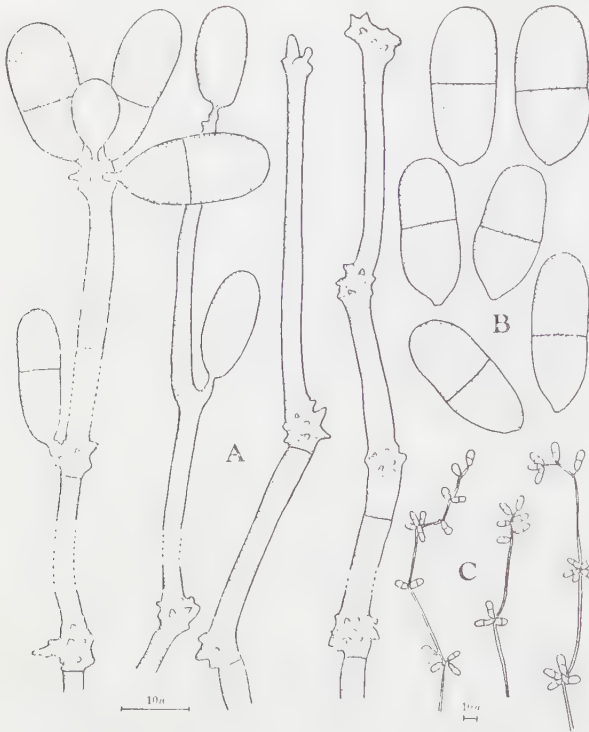


Fig. 2. *Arthrobotrys arthrobotryoides*
A. Conidiophores bearing conidia B. Conidia C. Habit

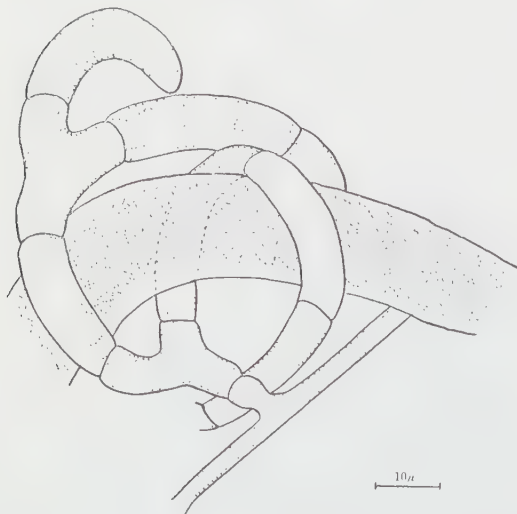


Fig. 3. Hyphal network of *Arth. arthrobotryoides* with a captured eelworm

Although this is taken as the member of the Zoopagaceae of Phycomycetes by some mycologists, the present author treats this as one of the imperfect fungi.

Arthrobotrys arthrobotryoides (Berlese) Lindau, in Rabenhorst, Krypt. Fl. Pilze 8: 371 (1907); Drechsler, in Mycologia 35: 145 (1944).

Growth on malt agar luxuriant, broadly spreading, loosely floccose, at first white, then becoming pale orange colour. Aerial hyphae well developed, branched, septate, 2.0–2.5 μ in diam., hyaline. Conidiophores erect from trailing hyphae, robust, septate, unbranched, 200–500 μ long, 4.0–6.0 μ in diam., inflated at apices and at points below the apices; each capitate node bears bluntly short sterigmata-like protuberances on which 5–10 conidia are produced, and the arrangement of nodes is not so regular, often shows sinuous appearance in the whole shape of conidiophores. Conidia didymous, with acute points at the base, oblong, slightly constricted at the septa; two cells are nearly of equal length, often the lower cell is slightly larger than the upper; (15) 20–23 × 9–10 μ, hyaline.

Predacious. Short lateral branches, measuring $4-6\mu$ in diam., are produced from the mycelial curl and form the hyphal loops. In these networks, eelworms are captured, killed and consumed as seen in *Arth. oligospora*.

Hab. On dead leaves of *Acer* sp., Bot. Gard., Sapporo, Hokkaido (Aug., 1956).

This species is peculiar in the arrangement of the capitate nodes of the conidiophores and also in the oblong conidia which consist of nearly equal sized two cells.

Dactylella attractoides Drechsler, in *Mycologia* **35**: 360 (1943).

Growth on malt agar, slow, hardly wrinkled, consisting of scarce aerial hyphae, pure white at first, then becoming pale pinkish coloured; after long time, aerial hyphae develop at rather marginal area of the colony producing conidia; reverse pale pinkish coloured.

Aerial hyphae slender, simple or rarely fasciculate, irregularly branched, $2.0-2.5\mu$ in diam., hyaline. Conidiophores scarcely produced on malt agar, but on oat meal agar inoculated with sterilized leaves of *Musa*, much sporulation can be observed. Conidiophores erect from aerial or ascending hyphae, straight, much septate, producing conidia at the apices of simple or branched conidiophores, and new growing points develop near the base of the previous conidia. The conidiophore measuring $2.5-3.5\mu$ in diam., therefore, becoming extensively ramose as the results of the pronounced irregularity of the branching. Conidia commonly 5-20 on each fructification, fusiform, occasionally clavate, straight or often somewhat curved, usually 10-12 celled, $30-80 \times 9-10\mu$, hyaline.

Hab. On dead leaves of *Musa basjoo*, Mejiro, Tokyo (Sept., 1956).

This species is peculiar among the members of *Dactylella* in its characteristic spore shape.

Sporobolomyces coralliformis Tubaki sp. nov.

In musto maltato sedimentum pelliculumque formantur. Pelliculum primo tenue rosaceum mucosum, deinde incrassatum tenax rugosum, e cellulis ballistosporis et hyphis compositum. Cultura in agarico maltato restricte expansa mucilaginis,



Fig. 4. *Dactylella attractoides*

A. Conidiophores with mature and immature conidia B. Conidia

mucosa vel farinacea, rugulosa, margine fimbriata, salmonea. Cellulae ovoideae vel ellipsoideae $6.5-10.5 \times 3.5-6.0 \mu$. Ballistosporae reniformes $7-8.5 \times 3.5-5 \mu$, pallide salmoneae, germinando promycelium singularis elongatum productae. Sterigmata ex promycelio lateraliter vel terminaliter productae numerosae (plerumque 5-8), corniformes raro bifidae $8-27 \mu$ longae $2-5 \mu$ crassae. Hyphae septatae ramosae, $3.5-7 \mu$ crassae. Chlamydo sporae sphaeroideae vel ovoideae $10-12 \mu$ in diam, hyalinae vel pallide fuscrescentes. Fermentatio nullus. Nitras kalicus assimilatur. In medio minerali cum alcohole aethylico non crescit.

In malt extract, thin, pinkish, pasty pellicle is formed which becoming thick, tough, heavily wrinkled at age. The liquid is at first turbid, and sediment is formed after a month. This pellicle consists of budding cells, irregularly formed sterigmata, ballistospores and true hyphae.

On malt agar, growth is rather restrict, mucilaginous, pasty or powdery, wrinkled,



Fig. 5. *Sporobolomyces coralliformis*

- | | |
|---------------------|--|
| A. Ballistospores | B. Coralloid sterigmata producing many denticles |
| C. Vegetative cells | D. Chlamydo spores |

with prostrating hyphae at marginal area of the colony, salmon-pink coloured; mirror images of the colony are obtained on the covers of inverted plates.

Cells are ovoid or ellipsoid, $6.5-10.5 \times 3.5-6.0 \mu$. Ballistospores reniform-lunate (kidney shape) $(7.0) 7.5-8.5 \times 3.5-4.0 (5.0) \mu$, hyaline and salmon pink in mass, germinate, sending out one elongated promycelium on which short sterigmata are borne. Ballistospores may bud producing blastospores, and also are produced from the promycelium on short sterigmata. Sterigmata are formed on cells, ballistospores and characteristically on lateral side of the promycelium in abundant, continuous or forked; (8) $10-20 (27) \mu$ long, $2.0-4.5 (5.0) \mu$ in diam. Hyphae are formed at rather marginal area of the colony, septate, branched, commonly $3.5-5.5 (7.0) \mu$ in diam. The tips of filaments may or may not bear the ballistospores. Abundant chlamydospores are formed, thick walled, sphaeroid or ovoid, $10-12 \mu$ in diam., hyaline or pale brown coloured.

No fermentation occurred.

Sugar assimilation: Glucose+, Maltose+, Galactose—, Lactose—, Saccharose—

Nitrogen assimilation: Potassium nitrate+, Ammonium sulphate+, Petone+,
Asparagine+

Ethanol as sole source of carbon: not grow.

At 37° : not grow.

Hab. Isolated from the fruit-body of *Exidia* sp., Mt. Ashiwada, Yamanashi Pref. (Oct., 1955).

Type specimen is preserved in the Nagao Institute, Tokyo, Japan. In culture of this fungus, pinkish coloured cells bud and produce numerous ballistospores which are reproduced by repetition. This cultural stage is closely agreeable to those of previously described *Sporobolomyces* (Tubaki, 1953). Sterigmata bearing ballistospores are commonly produced at the lateral side of the cells and sometimes irregularly prolonged like those of *Sp. roseus*. In addition to the above characters, this fungus also produces true hyphae from the marginal part of the colony. The hyphae are septate and somewhat sinuous, and produce rather long sterigmata. The curious feature of this fungus is to have numerous conspicuous sterigmata with single ballistospores. It is another peculiar character that abundant chlamydospores are produced, associated with many budding cells in old culture.

In producing true hyphae as well as in physiological characters, this fungus agrees closely with *Sp. salmonicolor*. Because of the presence of promycelium which forms pleurogenously many sterigmata (denticles), this can be clearly separated from *Sp. salmonicolor*. In the mode of production of chlamydospores, this is near *Sporidiobolus*, but differs from the latter in absence of clump-connection on hyphae. Therefore, the present fungus seems to be the intermediate form of *Sporobolomyces* and *Sporidiobolus*.

It is interesting that this fungus was isolated from the surface of fruit-body of *Exidia*. As stated by Martin (1952), the manner in which the ballistospores are formed is exactly that of the germination by repetition, characteristic of many species of Tremellales, and it may be pointed, though it is no more than a speculation, that

the Sporobolomycetaceae may be justifiable to be included provisionally among the Tremellales. Hereupon, it is interesting to compare the imperfect stage of *Exidia* with *Sporobolomyces*. From the result of the cultivation of some species of *Exidia*, however, there are no certain similarities between the cultivation of *Exidia* and *Sporobolomyces*.

No perfect stage of the present fungus could be obtained in the assorted culture with fourteen type-cultures of *Sporobolomyces* which are originated from Centraalbureau voor Schimmelcultures and Nagao Institute.

When several dilution transfers were taken on malt agar plates, two types of colonies were resulted. This fact is also common in the cultures of *Sporobolomyces* and *Sporidiobolus*. The original type is pasty, and then, the very mucous and non-mucous types appear as the result of cultivation on agar media. These two types of colonies are shown in the plates. The non-mucous (often powdery) type seems dominant with numerous chlamydo-spores, which are scanty in mucous type.

Because of the fact that the state of carotenoid-pigment does not always show the coloration of yeast as stated by

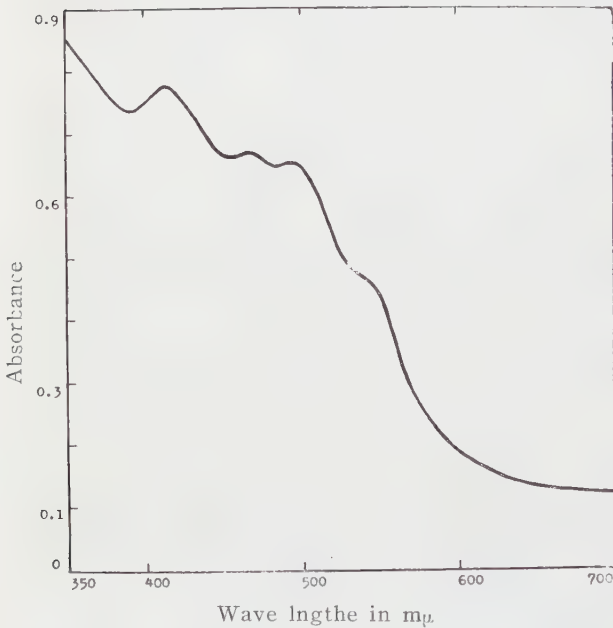


Fig. 6. Absorption spectra of living material of *Sporobolomyces coralliformis*

Dr. Nakayama (1954), the present author considers that at least the absorption spectra of the pigment must be added in the description of new yeast species, especially in red yeast. Accordingly, in the present study, the living material was examined spectrophotometrically in a Beckman DU Spectrophotometer at a spectral band width of 10μ by using the Shibata's method. Absorption maxima in this fungus were 410, 470 and 490 in millimicrons as shown in Fig. 6.

Much of this work was indebted to Dr. K. Kominami and Dr. Y. Kobayasi to whom

the writer owes thanks for their constant guidances. He is also indebted to Dr. K. Shibata, Tokugawa Institute for Biological Research in Tokyo, for pursuing the test on absorption spectra of *Sp. coralliformis*.

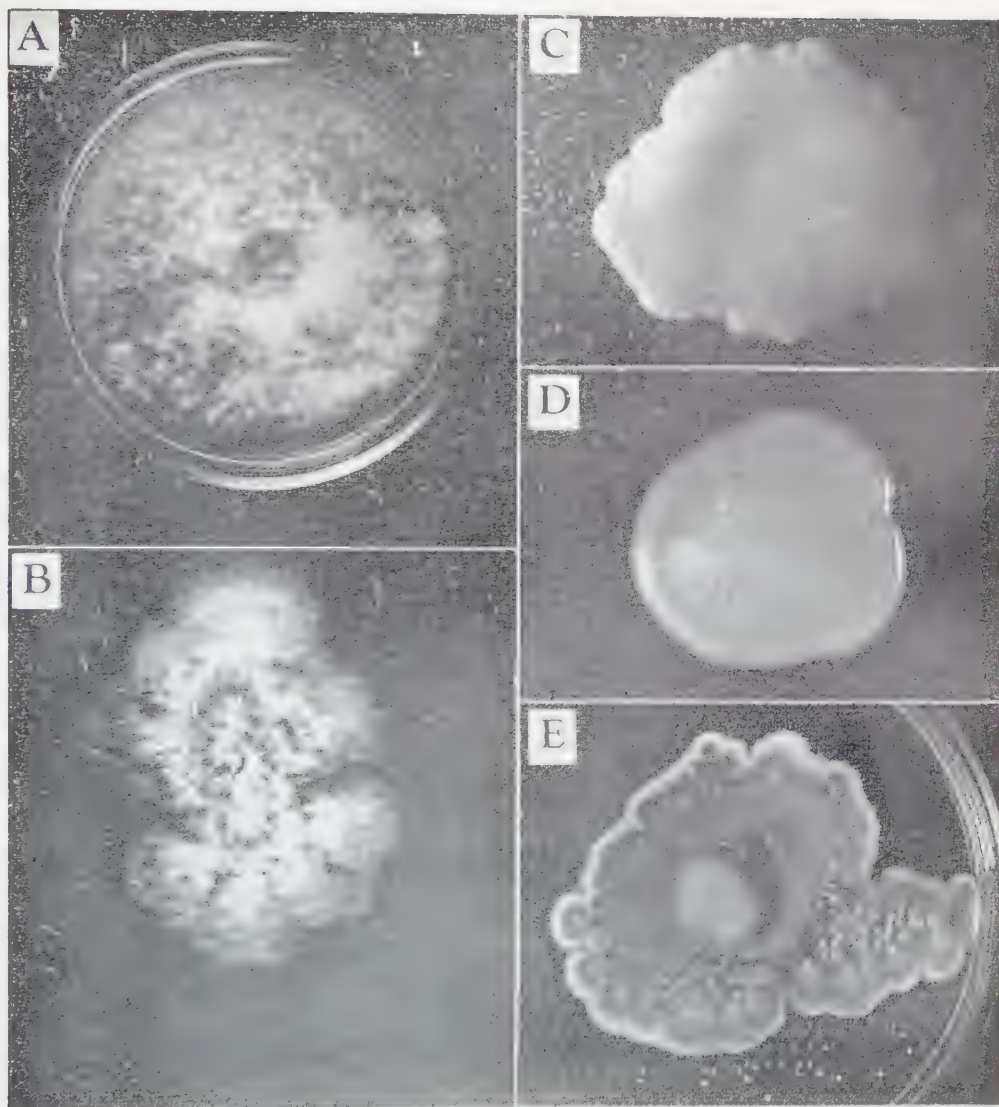


Fig. 7. A. *Arthrobotrys arthrobotryoides*, $\times 0.6$
 B. *Dactylella attractoides*, $\times 2$
 C-E. *Sporobolomyces coralliformis*
 C. original type, $\times 0.8$
 D. mucous type, $\times 1$
 E. non-mucous type, $\times 0.8$

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The Stigma Reaction III. Withering of the Stigma by Pollination in Gramineous Plants

by Kotaro WATANABE*

渡辺光太郎：柱頭反応 III. 受粉によるイネ科柱頭の凋萎

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It has been known in some orchids that the pollinated stigma cells as well as the pollinia themselves on the stigma are disorganized and turn brown in a few days after pollination (5, 6, 8). For example, in self-pollination of *Notylia*, the phenomenon occurs two days after pollination and the flower soon falls off (6). Fitting showed in his well-known work on "pollen hormone" (1,2) that pollination brings about visible, rapid changes of the various floral organs in orchids. It was also reported by Strasburger (8) that when the pollen grains of *Agapanthus umbellatus* and of *Nicotiana Tabacum* germinated on the stigma of *Achimenes grandiflora*, the stigmatic portions with which the pollen tubes had been in contact turned brown and perished. Also it was noted by Robinsohn (7) that the stigma of *Juglans* turned brown at the portion where the pollen tube had entered.

Recently an immediate change of stigma cells by pollination has been found by Kato (3) in *Secale cereale*. The stigma cells to which a pollen grain attaches are stained deeply with aceto-carmin or other aqueous solutions of various dyes, showing clear contrast with those free from pollen. This phenomenon (stigma reaction) was recognized by Kato and Watanabe (4) to be found among all gramineous plants observed: in intra-specific as well as inter-generic pollination. These amount, in intra-specific pollination, to 57 species, belonging to 37 genera, and 38 combinations in inter-generic pollinations (4).

In the course of the above study (4), the present author has observed, in each of the cases, withering of pollinated stigma cells¹⁾, one of the stigma reaction (3). The detailed observations of the withering phenomenon are the subject of this paper.

Materials and Methods

Barley, common wheat, rye, einkorn wheat, maize and rice were mainly used as materials for study. A mature feathery stigma taken out from a floret was placed on a slide glass and pollinated with fresh pollen directly from a dehiscing anther. In the case of preserved, shrivelled pollen, the pollination was done by means of a

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.
京都大学農学部応用植物学研究室

1) A part was read at the 15th general meeting of the Botanical Society of Japan in November, 1950, in Tokyo. cf. Bot. Mag. Tokyo 63: 223 (1950).

small hair-pencil. The preparation thus made was put in a Petri-dish and observed under a microscope at a certain time after pollination. In some cases, the preparation was immediately placed under the microscope and observed continuously from the beginning, in order to see all details of the withering process. Besides these, stigmas which were pollinated naturally in the field were also studied.

Observations

Behaviours of stigmas after pollination. When the florets of barley or wheat are emasculated in the field, their stigmas remain healthy in turgescient state for about ten days after castration, before some of them begin to wither. On the other hand, the stigma pollinated naturally in the field withers heavily in relatively short days after pollination. If we examine in greater details the florets which opened one or two days before, all of the pollinated stigma hairs or filaments are found to wither all along their length, whereas non-pollinated ones remain almost turgescient (Fig. 1 *b*).

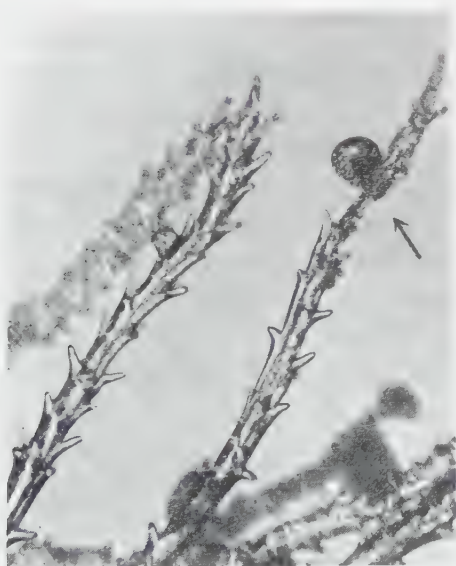


Fig. 1 *a*

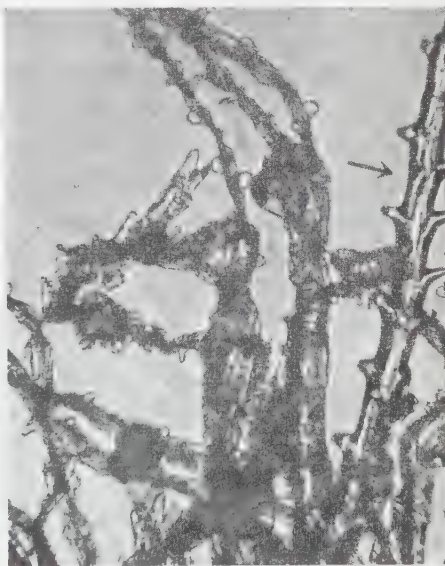


Fig. 1 *b*

Figs. 1 *a* and *b*. *Hordeum vulgare*. Withering of stigma filaments by natural pollination. Magnification, ca. $\times 220$.

a) The right stigma filament withers at the portion (\uparrow) to which a pollen grain is attached. The left, non-pollinated filament is entirely turgescient.

b) About two days after flowering. Non-pollinated stigma filament (\uparrow) is almost turgescient, while all pollinated ones wither heavily all over their length.

Withering begins at the stigma cell (or cells) to which a pollen grain is attached, and this withering spreads to the neighbouring cells along the stigma filament (Fig. 1 *a*). Due to the withering the affected portion of the stigma filament becomes narrower by one half to one fourth in width as compared with the normal one (Fig. 1 *b*). Accompanied with this, remarkable changes of nucleus in shape and structure occur in such a stigma cell, of which some description has been made by Kato (3).

The stigmas pollinated naturally in the field begin to wither usually a few hours after pollination, while withering progresses more or less rapidly under experimental conditions, where the detached stigma is used. For instance, in the case of common wheat, the stigma cells begin to wither in about 20 minutes after fresh pollen grains are shed upon. In rye and barley, such withering is clearly noticeable about 60 minutes after pollination. In non-pollinated stigmas placed on a slide it takes 6 to 7 hours and 1.5 to 2 hours, respectively, before the entire stigma withers. By pollination with shrivelled pollen which has been preserved in a parchment-paper in the room, however, the withering is recognizable about 20 minutes after the attachment of the pollen in rye and about 10 to 15 minutes in barley. These facts indicate that the withering occurs earlier by pollination with dehydrated shrivelled pollen grains than by pollination with turgescient fresh grains.

Behaviours of pollen grains and withering of stigma cells. Most fresh pollen grains exude liquid at their surface as soon as they are attached to the stigma (9). The exuded liquid immediately fills up the gap between the pollen and the stigma. Majority of pollen grains which are capable of liquid exudation germinate and send out the pollen tube into the stigmatic tissue. But some of the grains do not produce the tubes, but burst or shrink after exudation of liquid¹⁾. Some pollen grains fail to show such a liquid exudation, and begin to shrink from the beginning.

The process of withering of stigma cells is much affected by the manner by which pollen grains behave on the stigma cells. The initiation of withering may be arranged in the following order, with respect to the behaviour of pollen grains (Table 1).

Table 1. Relations between behaviour of pollen grains and beginning of the withering of stigma cells.

Order in beginning of withering of stigma cells		Behaviour of pollen grains
1	early	Exude liquid, and burst
2	↑ ↓	No exudation, shrink soon after pollination
3		Exude liquid, but do not germinate
4		Exude liquid, germinate, but a pollen tube stops to develop
5		Exude liquid, germinate, develop pollen tube to ovule
	late	

As shown in Table 1, withering of stigma takes place most readily when a pollen grain attaching to it bursts on the stigma cells, and less readily in the cells on which

1) In this case, the shrinkage of the pollen grain begins with the disappearance of the liquid between the grain and the stigma.

the pollen grain begins to shrink immediately. Withering of stigma begins still later in the case where a grain may still exude the liquid on the stigma but does not germinate or burst. There is no clear withering when pollen tubes are developing in the stigmatic tissue or in the style. In such a case the stigma cells wither distinctly after the pollen grains have sent out their contents completely and collapsed. In the stigma of einkorn wheat on a slide, for instance, the stigma cells, on which the pollen grains shrink or burst, show remarkable withering 20 to 25 minutes after attachment of pollen, when those with the turgid grains remain almost turgid. Similar observations were made in all species investigated.

These facts seem to indicate that bursting and shrinking of pollen grains have an intimate relation to the withering of stigma cells. This conclusion may also be supported by another observation. When a pollinated stigma of maize is taken out in the air after being kept in a moist chamber for 40 to 50 minutes, the stigma cells on which pollen grains burst begin to wither within 5 minutes. In about 10 minutes after the stigma was taken out from the chamber, some of the pollen grains on it shrink a little, while the others remain turgid. The stigma cells in the former case soon show slight withering, but those in the latter case begin to wither much later.

However, detailed observations revealed that in the case of attachment of several or many pollen grains, the slight withering of stigma cells starts before shrivelling of the stuck grains. Under these circumstances, the above-mentioned liquid between the pollen and the stigma has already disappeared. So long as the liquid exists, it is usual that the stigma cells do not wither. Accordingly, presence or absence of the liquid between the pollen and the stigma seems to have more intimate relation to the withering phenomenon than the shrinkage of pollen.

Withering of stigma cells by pollination with shrivelled pollen. Pollination with shrivelled pollen was undertaken, in order to investigate the withering of pollinated stigma cells in greater details. The longer the shrivelled grains are attached to the stigma, the stronger is the withering of stigma cells. Together with this action time, the grades of the withering may also depend upon the number of grains which are attached to a stigma filament and the area with which a pollen grain is in contact with a stigma cell. A stigma withers earlier in the portion where many pollen grains are attached than in that with one or a few pollen grains, and the withering goes slowly when a grain attaches to the top of a stigma cell. The withering spreads upward and downward from the point where a grain is attached until the stigma filament withers entirely all over its length; it proceeds faster upward than downward.

In maize, some of the shrivelled pollen grains, which have been collected from dehiscing anthers a few hours before pollination, swell on the stigma. A part of these swollen grains soon develop their tubes, but the others gradually shrink again with or without forming the nipple-like protrusion (9). It is sometimes observed in rye that

the stigma cells wither as soon as the shrivelled grains are attached to them and swell. Soon these swollen grains shrink again, perhaps because the withered stigma cells can not supply sufficient water for these grains to swell.

Discussion

In gramineous plants, physiological and morphological changes take place in the stigma cells soon after pollination. Of these changes (stigma reaction) the increase of stainability and withering of stigma cells to which pollen is attached are most conspicuous. In most cases these phenomena occur successively. Under certain conditions, however, the change of stainability is not followed by the nuclear change in shape¹⁾ and by withering of stigma cells. If a pollinated stigma is kept in a moist chamber, the stigma cells to which the pollen grains are attached are seen stained deeply by a dye solution, notwithstanding the fact that their nuclei do not show any change in shape. There is little withering of the stigma cells to which a pollen grain is attached, so long as the pollen tube is developing in the stigmatic tissue or in the style; in this case also the high stainability of stigma cells is distinguished, but this is not accompanied by the change of nuclear shape. These facts leads to the conclusion that the change of nuclear shape is brought about by the withering of stigma cells and that the withering can be distinguished from the change of stainability under certain conditions.

The withering spreads to the apical direction more rapidly than to the basal. This seems to be due to interruption of water supply from the basal part of the stigma filament in the regions of pollinated cells and their neighbours, which have already started to wither. It can frequently be seen that the stained cells are more numerous above the pollinated part of the filament than those below. The stigma cells which withered slightly, due to some reason or other, can not be stained with ease unless they receive pollen grains. But as the withering becomes evident, they are stained in a short time after staining, even in those cells which are not in contact with grains. The stainability of the cells may gradually be increased with the progress of withering.

The withering of stigma cells can be interpreted as being caused mainly by an increase in cell permeability of the pollinated stigma cells. In addition to this, the dehydrating power, exerted at the surface of the stigma cells, of the pollen grain may also be concerned with the phenomenon. On the other hand, presence of the liquid oozed out at the place where the pollen grain and the stigma come into contact makes the stigma cells begin to wither later. This may be interpreted as being due to prevention of water evaporation from the stigma cells at this place.

1) The nucleus of a stigma cell changes its shape from spherical to ellipsoidal or irregular after attachment of pollen (cf. Kato, 3).

Summary

1) Among various changes of gramineous stigma cells, caused by the attachment of a pollen grain, withering is one of the most conspicuous phenomena. The withering, beginning at the stigma cells to which a pollen grain is attached, gradually spreads to the neighbouring cells. Later the pollinated stigma filament withers entirely all over its length.

2) Pollen grains of the same anther show different behaviours when they fall on stigma cells. These different behaviours of the grains have an intimate relation to the beginning of withering of the stigma cells. In general, the withering begins first at the cells on which a grain bursts, then at the portion where a grain shrinks. Stigma cells on which pollen grains germinate and develop the pollen tubes wither latest.

3) The disappearance of the liquid, oozed out between the pollen and the stigma, has an intimate relation to the occurrence of withering. Some considerations were made on the withering phenomenon.

The author desires to acknowledge his great indebtedness to Prof. S. Imamura, for constant help and criticism throughout this study. He is also indebted to Mr. K. Kato, of the Botanical Institute, Faculty of Science, Kyoto University, for criticism and encouragement in carrying out this work.

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The Predetermined Polarity in *Porphyra* Monopores Shed from *Conchocelis*-thalli*

by Singo NAKAZAWA**

中沢信午**: アサクサノリのコンコセリス体から放出された単胞子に
おける先定的極性*

Received January 30, 1958

Introduction

Porphyra is a marine red alga cultivated for edible purposes in various districts along the coast of Japan. Its life cycle was a question especially as to how it passed the summer season and as to where its abundant spores, which gave rise to the winter fronds, came from in early autumn. The question has been solved by Drew (4), Kurogi (5), Kurogi and Hirano (6, 7), Suto *et al.* (12), and by Takeuchi *et al.* (13). Their investigations have revealed that the carpospores shed in spring from the frond, passed over the winter season, germinate on the shell and there they give rise to filamentous thalli called *Conchocelis* which pierce into the shell. Early in autumn, monospores are liberated from monosporangia developed in *Conchocelis*. The monospores, transported by move of sea water, adhere to the substratum, there they germinate to form winter fronds. Hereby, what is important in a technique of cultivation is to make the monospores much more abundantly adhere to the substratum. For this purpose particular investigations must be done on the mechanism of the adhesion. However, very little has been hitherto reported in this field except for some ecological investigations made by Suto (8-11), Suto *et al.* (12) and by Takeuchi *et al.* (13). Herein the writer presents some results of his cytological investigations on the monospores liberated from the *Conchocelis*-thalli.

Material and Method

The present experiments were carried out in October, 1956, at the Misaki Marine Biological Station of the University of Tokyo located at the tip of the Miura Peninsula, a little south from Tokyo. An abundance of monospores shed from the *Conchocelis* of *Porphyra tenera* cultured in oyster shells were used for the material.

To reveal the polarity differentiation, the material was stained vitally at various stages, from just after the liberation from *Conchocelis* to formation of young spore-

* Contributions from the Misaki Marine Biological Station of the University of Tokyo.

** Biology Department, Yamagata University, Yamagata, Japan. 山形大学生物学教室

lings composed of ten or so cells. The dyes used for the staining were auramin, aurantia, Bismarck brown Y, brasilin, brilliant green, Congo red, eosin, erythrosin Y, gentian violet, Janus green B, kernilin, methyl green, methylene blue, neutral red, Nile blue, pyronin, safranin, thionin, toluidin blue, and trypan blue. 0.1 per cent distilled water solutions of these dyes were diluted separately into filtered sea water of pH 8.1 in a proportion of one or two drops per 10 ml. The material was immersed into the staining media thus prepared contained in Petri dishes and observed with microscope. To make the spore germinate, they were made adhere to amylan threads of $30\ \mu$ in diameter by stirring the spores and the threads together in sea water irregularly by use of air bubbles.

Experiment and Observation

1) Spores just after being shed.

The fresh spore just after being shed from *Conchocelis* is about $12\ \mu$ in diameter. It is not of definite form but varies doing amoeboid movement over the substratum. After a while, if the spore remains without adhering to a substratum, the amoeboid movement ceases, the spore becomes spherical, a thick exosporium develops, and the spore enters the dormancy. Such a spore cannot adhere autonomically. The fate

of it is not clear. But the amoeboid spore, before entering the dormancy, can adhere firmly to the substratum autonomically. After having adhered, a morphogenetic movement makes the spore uprise to an ovate form, then it begins to germinate. The form alteration in time by amoeboid movement is illustrated in Figure 1.

Result of the vital staining at this stage is indicated in Table 1. A clear differential staining was observed in the amoeboid spore with Janus green, which is illustrated in Figure 2. That is, the cytoplasm was stained reddish purple at a part while at the other part it was stained blue. The cell wall was stained dark blue, and the plastid green, while the nucleus was not clear. The staining into different colours, appeared in the cytoplasm, implies a differentiation in a physical property. The direction of the staining differentiation was irrespective not only of the morphology of the amoeboid spore but also of the orientation of the field of the microscope. Therefore it appears that the differentiation is neither an influence of a special external condition nor what is related to the amoeboid movement, but it is a matter of intrinsic affair peculiar to each spore. It is specially noteworthy that this differentiation cannot be observed in the dormant spore, in which the cytoplasm is stained uniformly blue with the same dye, Janus

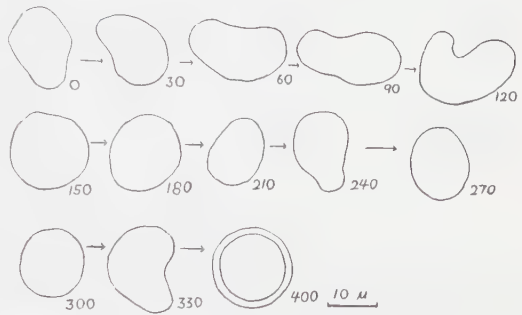


Fig. 1. Successive movement of an amoeboid spore and its final formation of the exosporium. Numerals indicate the pass of time in seconds.

Table 1. Vital staining of fresh spores before adhesion to the substratum

Dye	Staining				
	Amoeboid spore			Dormant spore	
	Cytoplasm		Periphery	Cytoplasm	Exosporium
	At a part	At the other part			
Brasilin	+	+	+	+	+
Congo red	—	—	—	—	—
Eosin	—	—	—	—	—
Janus green	+**	+*	+*	+*	+*
Methylene blue	—	—	—	—	+
Neutral red	+	+	+	+	+
Toluidin blue	+*	+*	—	+*	—
Control	—	—	—	—	—

* Stained blue, ** stained reddish purple (cf. Fig. 2)

green B. The amoeboid spore stained differentially ceases its amoeboid movement and dies sooner or later, so that its fate cannot be traced. That the cytoplasm differentiation is occurring before the spore adheres to a substratum is affirmed by a fact that the same differential staining is also observed in the spores floating in the staining medium. The exosporium of the dormant spore is stained red with Congo red, while the periphery of the amoeboid spore is not stained with the same. This also indicates that the amoeboid spore is deficient in the exosporium.

2) *Adhesion and the subsequent morphogenesis.* The amoeboid spore adheres to the surface of various substrata, and in such a state they are also stained with Janus green B. The adhered spores can be swayed at their distal end by moving the water by use of pipette. On this occasion, it is observed that the distal end is staining blue and the basal end, at which the spore is attached to the substratum, is staining reddish purple with Janus green B. Comparing this with the former experiment, it is affirmed that in the amoeboid spore a special part is prepared where the cytoplasm is stained reddish purple with Janus green B and at such a part the spore adheres to a substratum. That is, the point where the spore adheres to is predetermined.

Observation reveals that the spore, adhering at an end to a substratum, gradually uprises just like a tumbler transforming itself to an ovate form pointed towards the base and blunt towards the apex, the distal end (Fig. 3 B D). Then the plastid migrates to the apical half, and a vacuole develops at the base. Throughout this process, the basal cytoplasm is always stained reddish purple with Janus green B, while the apical half is stained blue (Table 2). Regardless of the differentiation in tint, the staining

always begins at the apical end and it spreads towards the base, so that the reddish purple staining at the base appears later than the blue coloration at the apex. This implies that there is a permeability gradient, highest at the apex.

3) *Segmentation of the adhered spore.* After completion of the morphogenetic movement, amoeboid to ovate, adhering to a substratum, the spore is cloven by a segmentation



Fig. 2. Amoeboid spores stained with Janus green B. Dots represent the part stained reddish purple.

Table 2. Vital staining of the adhered spore after occurrence of the morphogenetic movement (cf. Fig. 3).

Dye	Staining		
	Basal cytoplasm	Apical cytoplasm	Cell wall
Congo red	++	—	+
Eosin	++	—	+
Janus green B	+**	+*	+*
Neutral red	±	+++	+
Nile blue	—	+	—
Toluidin blue	+**	+*	—
Control	—	—	—

* Stained blue, ** stained reddish purple.

wall at right angles to the longitudinal axis (Fig. 3 E). Further segmentations take place successively parallel with the first and the each cell grows longitudinally, so that the sporeling becomes to be of a club-form (Fig. 3 F-H). Growing up to a stage composed of nine or ten cells, sub-apical cell or cells are cloven perpendicular to the former segmentations (Fig. 3 H). Sporelings were also stained vitally at various developmental stages. As a result, it was found that generally the basic dyes,

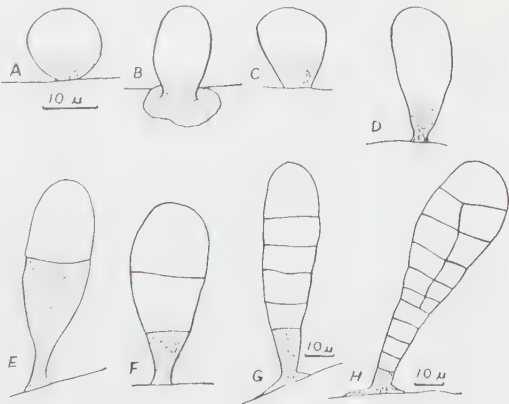


Fig. 3. Each stage from the adhesion to segmented sporelings. Dots represent the part stained reddish purple with Janus green B. A-F are of the same scale.

Table 3. Vital staining of the segmented sporeling (cf. Fig. 3).

Dye	Staining			
	Cytoplasm			Cell wall
	Basal cell	Subapical cells	Apical cell	
Auramin	—	—	+	—
Aurantia	—	+	+	+
Bismarck brown Y	—	+	+	+
Brasilin	—	+	+++	+
Brilliant green	—	—	—	—
Congo red	+++	—	—	±
Eosion	++	—	—	—
Erythrosin Y	+	—	—	—
Gentian violet	—	+	+	+
Janus green B	+**	+*	+*	+*
Kernilin	—	++	+++	+
Methyl green	—	+	+	—
Methylene blue	—	—	—	—
Neutral red	—	++	+++	+
Nile blue	—	+	++	—
Pyronin	—	+	++	—
Safranin	—	+	+++	—
Thionin	—	+	++	—
Toluidin blue	+**	+*	+*	—
Trypan blue	—	+	+++	—
Control	—	—	—	—

* Stained blue. **stained reddish purple.

except for Janus green B and toluidin blue, stained much more remarkably in apical cells than in basal cells, while the acidic dyes stained the basal cell selectively. Janus green B and toluidin blue stained both the apical and the basal cells. However, a staining differential in tint, as was observed in the former experiment, also did occur in sporelings with Janus green B and with toluidin blue. That is, each of these dyes stained blue in the apical part, while reddish purple in the basal cell, and this differentiation did not diminish for a long time.

4) *Abnormal sporelings.* Various abnormal sporelings were obtained caused by unknown factors. Some of them are specially noteworthy in relation to the present investigation. In some cases, the apical cell was basalized to form a pointed rhizoid-like cell at the tip (Fig. 4A). In such a cell, a large vacuole develops surrounded by acidophilic cytoplasm stainable with eosin, erythrosin Y, etc. like in the basal cell but different from in the usual apical cell. It is also stainable reddish purple with Janus green B. This cannot be due to death of the cell, because it is elongated by growth to a slender form different from the usual apical cell as indicated in Figure 4A. When this basalization does occur at the tip, the subapical cell becomes to take

the part of the apical cell and it is altered to be of a blunt form. Sometimes, basalization takes place at one of the subapical cells of a sporeling (Fig. 4B). In such a case, that cell becomes to be elongated to form a pattern like a basal cell and its cytoplasm turns to be acidophilic, and the cell neighbouring towards the base becomes blunt, so that the whole looks like a pile of two sporelings. This cannot be considered to have originated from junction of two different spores, as the basalized cell and its adjacent cell are connected with a common segmentation wall as well as they are involved in the same longitudinal cell wall.

Discussion

The selective staining of the basal cytoplasm with Congo red, etc. reveals its acidophilic properties. Contrarily, the apical cytoplasm is stained merely with basic dyes showing its basophilia. Janus green B, a Gr  bler's production, is a special dye which stained the apical cytoplasm blue but the basal cytoplasm reddish purple. This seems to be due to the reduction of Janus green B to the dimethyl safranin in the basal cytoplasm. The same reduction of Janus green B in living cells has been reported in some animals (1-3). Besides the same can be experimented in a test tube by adding a reductive agent such as sodium hydrosulfite. Toluidin blue also stained the basal cytoplasm purple and the apical cytoplasm blue, while its mechanism is not always clear. However, as to the staining with Janus green B, the matter seems to be related to the partial reduction of the dye in the basal region as above mentioned. The same differentiation can be traced back to the amoeboid stage immediately after being shed from the *Conchocelis*. That is to say, the spore is predeterminedly differentiated into two parts, i.e. a part at which the cytoplasm is reductive and is stained reddish purple with Janus green B, and the other part at which the cytoplasm is non-reductive and is stained blue with the same dye. The amoeboid spore adheres to the substratum at the reductive part as was observed in the second experiment and was illustrated in Figure 3. The adhesion, therefore, seems to be related to this differentiation. Actually, the dormant spore which does not show this differentiation can not adhere to a substratum. The same differentiation, i.e. the reductive properties at the base, is retained throughout the course of the sporeling development. However, the differentiation into the acidophilic base and the basophilic apex appears later than the morphogenetic movement of the adhered spore. When the apical or one of the subapical cells is basalized in form caused by unknown factors, its cytoplasm also turns to be of the same property as that of the basal cell although it is not in contact



Fig. 4. A) Basalization at the apical cell, B) the same at a subapical cell. Dots represent the part stained reddish purple with Janus green B.

with the substratum. This indicates that the acidophilic property and the reddish purple staining with Janus green B specific to the basal cytoplasm is an essential property of the basal cell regardless of the adhesion to a substratum.

Summary

The monospores of *Porphyra tenera* were experimented at various developmental stages from immediately after being shed from the *Conchocelis*-thalli to segmented sporelings. As a result, the following was revealed.

(1) At the amoeboid stage the cytolasm is stained with Janus green B reddish purple at a part and blue at the other part. This differentiation is observed before its adhering to the substratum. The spore tends to adhere at the part stained reddish purple.

(2) The adhered spore is stained with Janus green B also reddish purple at the base and blue at the apex. After the segmentation, merely the basal cell is stained reddish purple selectively but the apical cells blue with the same dye.

(3) The reddish purple coloration of Janus green B at the base seems to be attributed to a reductive property of the basal cytoplasm, and it is predeterminedly differentiated in the amoeboid spore.

(4) The basal cytoplasm which occupies the basal cell of the sporeling is acidophilic in nature and is stained selectively with Congo red, eosin, etc., while the apical cytoplasm is rather basophilic and is stained with basic dyes.

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Necessity of Auxin for the Growth Effect of Gibberellin*

by Gentaro KUSE**

久世源太郎**： ジベレリンの生長促進効果に対するオーキシンの役割*

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Ever since the discovery of gibberellins, they have been investigated with much interest in Japan and recently in the western countries, especially in America and England, as reviewed by Stowe and Yamaki (15). Gibberellins are negative in any of the commonly used auxin tests (7, 16), and auxin cannot substitute for gibberellic acid in making some dwarf plants grow tall (1). However, the relation of auxin to the action of gibberellins has not been studied precisely.

In the course of the author's investigation on the correlative growth, it was found that the gibberellin used (a mixture of A_1 and A_3) did not promote growth significantly in the absence of natural auxin or indoleacetic acid. If this be the case, results of experiments to determine the effect of gibberellins may not be well reproducible unless due care is paid for the auxin factor. This consideration may be important when one is trying to find bioassay methods for gibberellins, and when one studies the action mechanism of gibberellins. Here are reported the experiments which led to the above mentioned finding.

Material and Methods

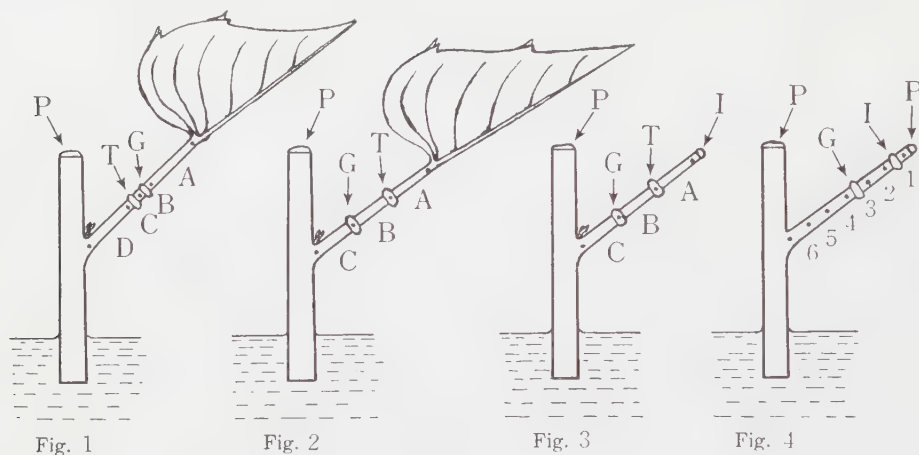
Single-node cuttings of young growing stems of sweet potato (*Ipomoea Batatas*, var. Norin No. 1) were used in the experiments. The node used was the one bearing the oldest of the immature folded leaves, of which the midrib and the petiole were about 4.5 and 3 cm. long, respectively, on the average at the beginning of the experiments. Materials were selected so as to be as uniform as possible, especially for the length of petioles. The stem was cut at 2 cm. above and 3 cm. below the node, the piece thus being 5 cm. long. The apical cut end of the stem piece was smeared with plain lanolin, and the basal end was put in a salts solution, as seen in Fig. 5. Pieces fixed in this way were kept in a wooden cabinet for the dark experiment, or, otherwise, under diffuse day light at about 1.5 m. apart from a window.

The lanolin pastes used were 0.5% gibberellin (GB) (a mixture of 55.6% A_1 and 28.6% A_3), 0.1% acid form of indoleacetic acid (IAA) and 2% sodium salt of 2,3,5-

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** Department of Botany, Faculty of Science, Kyoto University, Kyoto, Japan. 京都大学理学部植物学教室

triiodobenzoic acid (TIBA). They were smeared around the petiole to form rings about 2 mm. broad.



Figs. 1, 2, 3 and 4. Diagrams showing the zones for the elongation measurement and the sites of lanolin paste application in Experiments 1, 2, 3 and 4, respectively. P, plain lanolin; T, TIBA; G, GB; and I, IAA.

At the beginning of the experiments, Indian ink dots were put on a side of the petiole to measure the elongation of different parts of the petiole, as shown in Figs. 1 to 4. Elongation was represented mostly in percentage of the initial length of petiole or its part.

Results

Experiment 1. In his previous reports (10, 11), the author concluded that TIBA blocked, at the site of its application, the basipetal translocation of natural auxin and IAA. And the conclusion was confirmed by Niedergang-Kamien and Skoog (13), Niedergang-Kamien and Leopold (12), Hay (3) and Zwar and Rijven (18). The present experiment was planned for the purpose of seeing whether the growth effect of GB was also blocked by TIBA or not.

The petiole of each cutting was divided with Indian ink dots into four zones, which were named A, B, C and D successively downwards, as illustrated in Fig. 1. The zones A and D were about 15 mm. long and B and C were 3 mm. each at the beginning of the experiment.

Stem cuttings were divided into five groups according to presence and absence of the leaf blade and the treatments at the zones B and C, as shown in I~V, Table 1. The cuttings were kept in darkness for eight days.

The group II, Table 1, represents the growth pattern of the control, carrying the leaf blade and not subjected to any growth regulator treatment. The growth of the petiole is very much limited when the leaf blade has been excised (I, Table 1). When TIBA was applied at the zone C (III, Table 1), the growth of this zone and, more

Table 1. Effect of TIBA on the GB effect, GB- and TIBA-pastes being applied at B zone and/or C zone (see Fig. 1). Elongation in the dark in 8 days following the treatment made on Sept. 15, 1957. Mean of 5 samples.

Leaf blade	Excised	Intact			
Group	(I)	(II)	(III)	(IV)	(V)
Treatment at					
Central upper zone (B)	None	None	None	GB	GB
Central lower zone (C)	None	None	TIBA	None	TIBA
Elongation of	%	%	%	%	%
Apical zone (A)	10	40	173	123	217
Central upper zone (B)	10	90	133	G*→230	G*→190
Central lower zone (C)	10	120	T*→ 63	223	T*→ 80
Basal zone (D)	8	100	7	74	6
Entire petiole	9	76	91	119	115

* T and G indicate the sites of TIBA and GB treatments, respectively.

conspicuously, that of the lower zone, D, were inhibited. The growth of the zones above the treatment increased, possibly as a compensatory growth. These results are similar to those reported before (11). The natural auxin coming from the leaf blade is considered to be blocked by TIBA.

When the zone B was treated with GB, all the zones A, B and C elongated remarkably (IV, Table 1). The growth of D was smaller than in control (II, Table 1), perhaps because too much growth material was used up in the upper parts. By the application of TIBA just below the GB treatment (V, Table 1), the effect of GB was completely inhibited in the zones at and below the TIBA application. The uppermost zone elongated, possibly by compensation, so much as to make the elongation of the entire petiole comparable to the case of the group IV. Thus, TIBA inhibited the growth of the zones at and below the site of its application, both when GB was, and was not applied (V and III, Table 1).

Two alternative explanations may be suggested for the effect of TIBA blocking the downward propagation of the GB effect. Namely, (a) the translocation of GB is inhibited by TIBA, just as natural auxin and IAA are, and (b) the translocation of GB is not blocked, but its growth-promoting effect is inhibited at and below the site of TIBA application. The following experiments were undertaken in order to investigate the relations of TIBA, GB and auxin in the elongation of sweet potato petiole.

Experiment 2. The petiole was divided into three parts of equal length, as illustrated in Fig. 2. Pastes were smeared around the petiole at the boundaries between zones. Other conditions were the same as in the preceding experiment.

Effects of single applications of TIBA (III, Table 2) and GB (IV, Table 2) were the same in general trend as in the foregoing experiment. In the present experiment, TIBA was applied above the site of GB application (V, Table 2). And it was

found that GB was quite ineffective below the TIBA treatment. TIBA is believed to block the downward travel of auxin. Hence it was suspected that GB could not cause its growth effect in the absence of auxin.

Table 2. Effect of TIBA blocking the downward propagation of the effects of the leaf blade and of GB. Elongation in the dark in 7 days following the treatment made on Sept. 18, 1957. Mean of 5 samples.

Leaf blade		Excised		Intact		
Group		(I)	(II)	(III)	(IV)	(V)
Treatment at the part						
One third from the tip		None	None	TIBA	None	TIBA
One third from the base		None	None	None	GB	GB
Elongation of		%	%	%	%	%
Apical zone	(A)	8	51	135	102	151
				T*→		T*→
Central zone	(B)	7	125	13	197	10
					G*→	G*→
Basal zone	(C)	6	96	6	107	5
Entire petiole		7	91	50	134	56

* See Table 1.

Experiment 3. Effect of the leaf blade on the petiolar growth may involve effects by the natural auxin and by the so-called food factor. In order to exclude the latter effect, the leaf blade was excised and 0.1% IAA-paste was smeared on the cut surface, as seen in Fig. 3. The experimental procedures were the same as in Experiment 2, except that the cuttings received diffuse sun light during the daytime. The elongation in eight days was measured.

Table 3. Effect of TIBA blocking the downward propagation of the effects of IAA and of GB. Elongation in the diffuse light in 8 days following the treatment made on Sept. 19, 1957. Mean of 5 samples.

At the cut tip of petiole		Plain lanolin	IAA-lanolin			
Group		(I)	(II)	(III)	(IV)	(V)
Treatment at the part						
One third from the tip		None	None	TIBA	None	TIBA
One third from the base		None	None	None	GB	GB
Elongation of		%	%	%	%	%
Apical zone	(A)	8	37	71	106	103
				T*→		T*→
Central zone	(B)	8	76	10	142	9
					G*→	G*→
Basal zone	(C)	5	41	5	86	5
Entire petiole		7	52	28	114	40

* See Table 1.

The results as given in Table 3 indicate that the general trend of the effects of TIBA and GB was the same as in Table 2, only the amount of growth was smaller in the present cases. The growth seemed to be limited by the lack of the food factor to come from the leaf blade. But the effects of TIBA and GB on the growth pattern were the same, whether the leaf blade was present or not. Hence the food factor does not seem to be modified by TIBA or GB. Fig. 5 shows a representative set of cuttings at the close of the experiment.

It became highly probable from this experiment that the factor, which was blocked by TIBA and in absence of which GB could not cause its growth effect, was auxin. Next experiment was planned in order to confirm the necessity of IAA for the GB effect.

Experiment 4. Experiment was designed so as to discriminate the effect of GB or IAA from that of the coincidence of the two. The leaf blade was excised and the cut surface of the petiole was smeared with plain lanolin. A petiole was divided into six 5 mm. zones to be numbered as shown in Fig. 4.

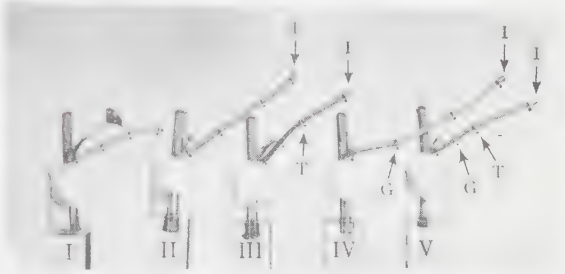


Fig. 5. A representative set of cuttings from the experiment summarized in Table 3, photographed at the close of the experiment. Roman numerals correspond to those in the table. T, G and I indicate TIBA-, GB- and IAA-pastes, respectively.

Table 4. Elongation of the entire petiole (of 30 mm. original length) following the first treatment (made on Sept. 21, 1957) and following the second treatment made 3 days after the first. *Italicized values* represent the elongation in 1, 2, 4 and 8 days after the second treatment. (1z) and (3z) represent that the zones numbered 1 and 3, respectively, were treated. Mean of 5 samples.

	(I)		(II)		(III)		(IV)	
First treatment	GB (3z)		GB (3z)		None		None	
Second treatment	None		IAA (1z)		IAA (1z)		GB (3z) & IAA (1z)	
Days after the first treatment	mm	mm	mm	mm	mm	mm	mm	mm
3	2.34	—	3.54	—	1.90	—	1.80	—
4 [1]*	2.62	<i>0.28</i>	15.56	<i>12.02</i>	11.82	<i>9.92</i>	12.00	<i>10.20</i>
5 [2]*	2.62	<i>0.28</i>	20.94	<i>17.40</i>	16.40	<i>14.50</i>	18.06	<i>16.26</i>
7 [4]*	2.62	<i>0.28</i>	23.34	<i>19.80</i>	18.82	<i>16.92</i>	21.76	<i>19.96</i>
11 [8]*	2.62	<i>0.28</i>	23.76	<i>20.22</i>	19.58	<i>17.68</i>	21.92	<i>20.12</i>

* Days after the second treatment.

One half of the cuttings were treated with the GB-paste at their zone No. 3, and the rest were not. The elongation of the treated petiole (groups I and II, Table 4) was only a little larger than the untreated ones (III and IV) in three days. Then,

one half of the GB-treated petioles were treated by IAA-paste at their zone No. 1 (II, Table 4), the rest being left as they were (I, Table 4). The IAA-treated petioles elongated very much in one day following this second treatment, while those without the IAA treatment elongated little. As days passed the former grew farther on, while the latter not at all.

Of the petioles not treated by GB at the beginning of the experiment, one half (III, Table 4) were treated on the fourth day with the IAA-paste at the zone No. 1, and the rest (IV, Table 4) were doubly treated on the same day with GB at the zone No. 3 and IAA at the zone No. 1. Within a day following these second treatments, the petiole of either group elongated conspicuously, at roughly the same rate. But later, the double treatment (IV, Table 4) resulted in a larger elongation than the single IAA application (III, Table 4). And the growth of group IV after the second treatment

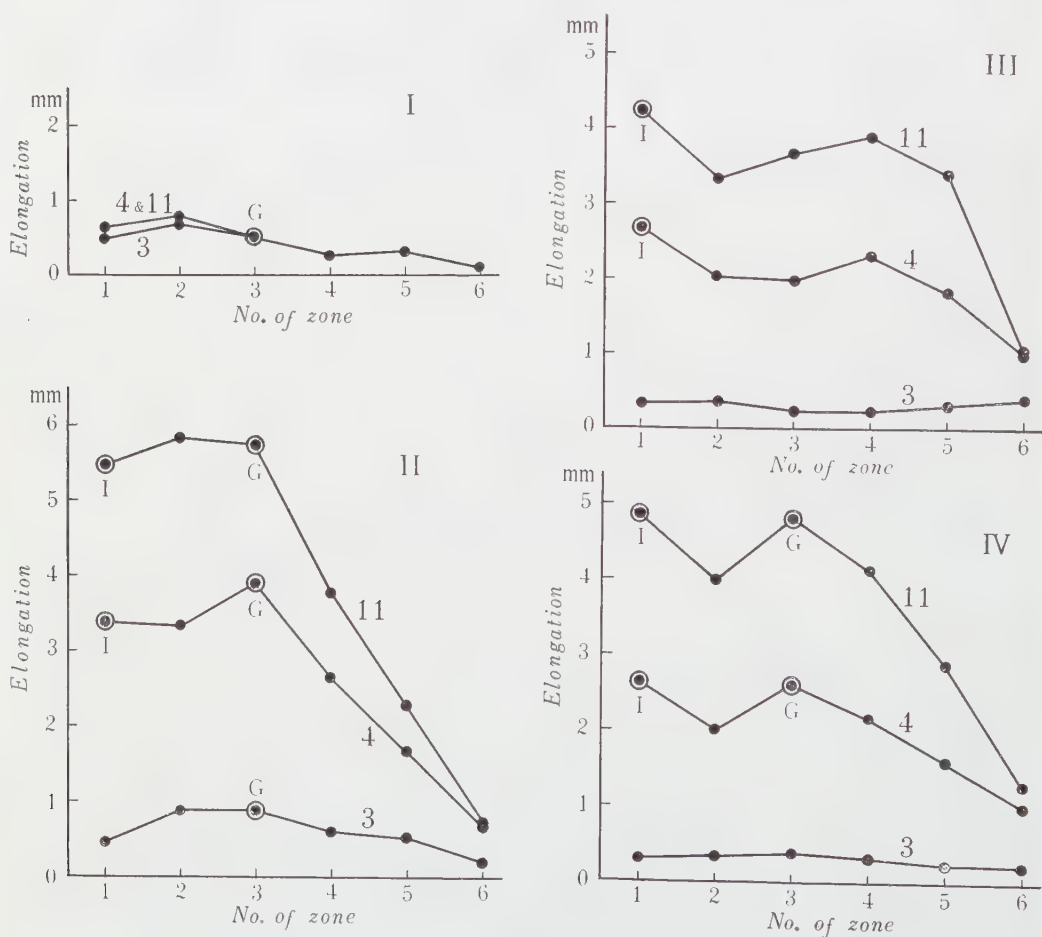


Fig. 6. Distribution of growth along the petiole of which the entire elongation is presented in Table 4. I, II, III and IV correspond to the same numbers in the table. The curves 3, 4 and 11 represent, respectively, the elongations measured 3, 4 and 11 days after the first treatment, the length of each section being 5 mm. at the time of this treatment. G and I on the curves indicate the sites of applications of GB and IAA, respectively. Mean of 5 samples.

almost overtook that of group II finally.

The group I shows that GB alone cannot cause any significant elongation in the petiole under the experimental conditions. But in the presence of IAA (II and IV, Table 4), the growth-promoting effect of GB becomes apparent, the growth in the presence of the two together surpassing that in the presence of IAA alone (III, Table 4).

The distribution of elongation along the petiole is illustrated in Fig. 6. Young petioles, such as those used in the experimental, elongate most conspicuously at and near the zone No. 4, either when their leaf blades are intact or when these are excised and substituted by IAA. But that zone did not grow better than others when no auxin was supplied, as shown by curves 3 of III and IV in Fig. 6. GB promoted the growth at or near the zone of application (curve 3, II, Fig. 6). This slight effect of GB may be conditioned by the residual natural auxin.

The zone No. 4 made a peak of growth also in the petiole treated with IAA at the zone No. 1 (curve 11, III, Fig. 6). In this case the zone of the treatment elongated conspicuously. When GB was applied at the zone No. 3, in addition to IAA at No. 1, the zones from No. 1 through No. 3 elongated very prominently (curves 4 and 11, II and IV, Fig. 6). In these cases, the lower half elongated as much as, or a little less than, in the case of the single application of IAA. Perhaps the growth of the lower half may be limited by growth material.

The debladed cutting has the material sufficient to support so much petiolar growth as represented by II, Table 4. Hence the growth in I and III (Table 4) must be limited respectively by IAA and GB. The total growth in IV is a little less than in II (Table 4), perhaps because GB is slow to be fully active. This is suggested by comparing the curves 4 of II and IV, Fig. 6, GB in the former case having penetrated in the tissue when IAA was applied.

Discussion

It has been found by Kato (7, 8, 9) and other investigators (4, 16) that GB is a growth regulating substance of the nature quite different from auxin. Experiments reported above also substantiate it. However, more important finding by them is that the growth-promoting effect of GB does not come about unless auxin is present in the tissue.

The slight elongation of the petiole treated with GB alone (I, Fig. 6) may be due to the presence of auxin remaining in the petiole. In spite of the food reserves which could support the petiole to grow nearly 80% of its original length (cf. II and IV, Table 4), the petiole did not practically grow after the fourth day. Auxin may have disappeared in the petiole.

If the "no auxin, no GB-effect" principle is correct, it is natural that GB is inactive in the standard auxin assays (7, 16), in which the natural auxin in the test tissue is minimized so as to make the reaction to the applied auxin apparent.

Hayashi and Murakami (4) found that pea epicotyl sections which had been

starved in distilled water lost their response to the single application of GB, while they were sensitive to auxin. They also found that L-tryptophane increased the growth response of pea sections to GB (4). These facts are favourable for the above-mentioned principle. The synergism between GB and auxin has been noticed (6, 9, 14). Here, if it is admitted that auxin is prerequisite to the GB-effect, the interpretation of the interaction between auxin and GB becomes easy. Reliable bioassay methods might be found if the auxin factor is taken into consideration.

Brian et al. (2) maintained that GB must be considered to be an auxin in the sense of the definition of Tukey et al. (17), though they admitted that GB failed to elicit many of the physiological responses characteristic of auxin. If one sticks to the definition which was proposed before GB was studied intensively, he will face to much inconvenience in discussions such as on the problem of the necessity of auxin for the activity of GB. On the other hand, no fruitful researches seem to be led by merely classifying GB as auxin. If GB should be named an auxin for some reason or other, modifiers are needed which discriminate GB from the classical auxin, as Kato claimed (9).

Summary

1) Using single-node cuttings of young sweet potato stems, elongation of petiole was observed after the treatments with gibberellin (GB, mixture of A_1 and A_3), indoleacetic acid (IAA) and 2, 3, 5-triiodobenzoic acid (TIBA) as lanolin pastes.

2) GB promoted the elongation of petiole when the leaf blade was present, or was excised but replaced by IAA-paste.

3) TIBA inhibited the growth-promoting effect of GB, as well as of IAA, to appear below the site of its application.

4) Debladed petiole elongated but little even if GB-paste was applied to it. However, when IAA was supplemented later, it elongated more than the one which was supplemented with IAA but had not received GB beforehand.

5) From these results, it is concluded that GB cannot produce its growth-promoting effect unless the tissue contains natural auxin or exogenous IAA.

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Physiological Studies on Growth and Morphogenesis of the Isolated Plant Cell Cultured *in vitro* IV.

The Effects of Some Vitamins, Plant Growth Hormones and Fat Soluble Substances

by Tadashi SANDAN*

山段 忠*: 遊離植物細胞の生長成形に関する生理学的研究 IV.
ビタミン, 植物生長ホルモンおよび脂溶性物質の影響

Received February 12, 1958

In the previous papers (1955, 1956) the author reported that an isolated internodal cell of *Nitella* and even a cell fragment obtained from the internodal cell by strangulation were able to grow and develop a new shoot and rhizoids when they were cultured in agar gel with suitable culture solutions. Furthermore, the effects of pH, IAA and metabolic inhibitors upon the morphogenesis of the cell were observed (Sandán and Ogura, 1957). The present report deals with the effects of some vitamins, plant growth hormones and fat soluble substances on the morphogenesis of an isolated internodal cell of *Nitella in vitro*.

Material and Method

An isolated internodal cell of *Nitella flexilis*, which was about 3.0 cm. in length and 400 μ in width, was used as material. The material were cultured in the vertical, normal position in a test tube filled by half with 0.6% agar gel according to the method described in the previous paper (1955). In the present work, solutions of α -naphthaleneacetic acid, (NAA), β -indolebutyric acid (IBA) and 2,4-dichlorophenoxyacetic acid (2, 4-D) in various concentrations were applied as growth hormone. Furthermore, solutions of vitamin B₁, B₂, B₆, B₁₂, C and nicotinic amide in various concentrations were used as vitamin, and also dilute solutions of acetone, ether and alcohols were tested. In the cases of growth hormones, Sørensen's phosphate buffer solution (M/100, pH 6.6) was used as the basic culture solution (control), and distilled water was applied as control in all the other cases. Especially, in the cases of fat soluble

* Biological Institute, Kyoto Liberal Arts University, Fukakusa, Kyoto, Japan. 京都学芸大学
生物学教室

substances, the test tubes containing material were covered with glass slip and the space between the tube and slip was filled with lanolin in order to prevent evaporation of the fat soluble substance. For the removal of fungi and bacteria from the culture medium, 0.3 mg. of Trichomycin P tablet and 0.1 mg. of streptomycin were added to 500 ml. of the culture solution. All experiments were carried out at room temperature under diffuse light of about 80 lux. As to the effects induced by these reagents upon the morphogenesis of the cell, the author paid his attention to the next two points: first, the effect on the formation of a shoot and rhizoids from the cell, and second, the effect on the elongation of the shoot and rhizoids. The former is represented as the time required by the material for shooting and forming rhizoid after it was brought into the culture medium and the latter is measured as the length of shoot or rhizoid 15 days after appearance. The values shown in the tables are the averages of five experiments in each case.

Results

1 Effect of plant growth hormones

The effects of NAA, IBA and 2,4-D on the morphogenesis of the cell are summarized in Table 1. According to the table, NAA and IBA accelerated the morpho-

Table 1. Effect of plant growth hormones on the morphogenesis of the cell

	Conc. (mg./l.)	Time for shooting (days)	Length of shoot (cm.)	Time for rhizoid formation (days)	Length of rhizoid (cm.)
Control		16	1.2	16	1.5
NAA	0.25	16	1.2	16	1.5
	0.50	16	1.2	15	1.5
	0.80	15	1.3	15	1.9
	1.00	16	1.2	12	1.8
	1.50	16	1.2	11	1.8
	2.00	16	1.2	13	1.6
	5.00	17	1.1	15	1.5
	10.00	18	1.1	16	1.3
	30.00	23	0.4	20	0.3
	40.00	no shooting	—	no rhizoid formation	—
IBA	0.20	16	1.2	16	1.5
	0.50	16	1.2	16	1.5
	1.00	12	1.7	15	1.8
	1.50	13	1.6	15	1.7
	2.00	15	1.3	13	1.6
	5.00	16	1.2	15	1.6
	10.00	16	1.2	15	1.6
	20.00	16	1.1	18	1.1
	40.00	23	0.2	23	0.6
	50.00	no shooting	—	no rhizoid formation	—
2,4-D	0.01	16	1.2	16	1.6
	0.02	15	1.2	16	1.6
	0.05	16	1.2	16	1.5
	0.10	16	1.1	19	1.4
	0.50	18	0.9	20	1.1
	1.00	20	0.8	20	0.9
	5.00	23	0.5	24	0.3
	10.00	26	0.4	28	0.2
	20.00	no shooting	—	no rhizoid formation	—

genesis of the cell in low concentrations and the optimum concentration for shooting was different from the one for rhizoid formation, but the optimum concentration for elongation of shoot was the same as the one for elongation of rhizoid in all cases. NAA seems to be more favourable for rhizoid formation than for shooting.

2 Effect of vitamins

Vitamin B₁, B₂, B₆, B₁₂, C and nicotinic amide all accelerated the morphogenesis of the cell in low concentrations, and above all, vitamin B₂ and C were more favourable than the others. The fact that the optimum concentration for shooting is different from that for rhizoid formation is also observed here as it has already been stated in the case of growth hormones. The maximum concentration of vitamins above which the cell could not survive and the range of concentration in which the morphogenesis of the cell was promoted are as follows: vitamin B₁, 30 mg./l. (8-16 mg./l.); vitamin B₂, 30 mg./l. (10-16 mg./l.); vitamin B₆, 24 mg./l. (6-10 mg./l.); nicotinic amide, 20 mg./l. (2-8 mg./l.); vitamin B₁₂, 18 mg./l. (2-4mg./l.); vitamin C, 22 mg./l. (2-8 mg./l.).

Below these accelerating concentration ranges the morphogenesis of the materials was not different from the one in control. The results in the case of treatment with the most suitable concentration of each vitamin for shooting and for rhizoid formation are summarized in Table 2.

Table 2. Effect of vitamins in appropriate concentrations on the morphogenesis of the cell

	Conc. (mg./l.)	Time for shooting (days)	Length of shoot (cm.)	Time for rhizoid formation (days)	Length of rhizoid (cm.)
Control		16	1.29	16	1.57
Vitamin B ₁	10	14	1.32	16	1.91
	12	15	1.31	14	1.83
Vitamin B ₂	10	12	2.18	13	2.48
	14	13	2.13	11	2.42
Vitamin B ₆	6	13	1.46	13	2.26
	8	14	1.41	12	2.27
Nicotinamide	5	12	1.48	13	2.19
	6	13	1.39	12	2.21
Vitamin B ₁₂	2	14	1.36	15	1.64
	3	14	1.28	13	1.62
Vitamin C	4	12	2.19	13	2.38
	6	13	2.13	12	2.26

3 Effect of fat soluble substances

All fat soluble substances so far used checked the growth of the cell in high concentrations and had no effect in low concentrations but intermediate concentrations, which were relatively low, accelerated the morphogenesis of the cell. The maximum concentration of fat soluble substances above which the cell could not survive and the range of concentration in which the morphogenesis of the cell was promoted are as follows:

methanol, 0.6 M (0.05–0.15 M); ethanol, 0.7 M (0.05–0.1 M); propanol, 0.7 M (0.05–0.1 M); butanol, 0.5 M (0.02–0.05 M); ether, 0.15 M(0.05–0.1 M); acetone, 0.7 M (0.05–0.2 M).

The results in the case of treatment with the most suitable concentration of each fat soluble substance for morphogenesis of the cell are summarized in Table 3. Noticeably, the optimum concentration for shooting was the same as the one for rhizoid formation and also as the one for elongation of shoot or rhizoid in the cases of all fat soluble substances so far used.

Table 3. Effect of fat soluble substances in appropriate concentration on the morphogenesis of the cell

	Conc. (M)	Time for shooting (days)	Length of shoot (cm.)	Time for rhizoid formation (days)	Length of rhizoid (cm.)
Control		16	1.28	16	1.58
Ether	0.1	14	1.56	13	1.93
Acetone	0.05	13	1.64	13	1.84
Methanol	0.1	14	1.54	15	1.61
Ethanol	0.1	15	1.53	15	1.63
Propanol	0.05	15	1.51	15	1.57
Butanol	0.02	16	1.48	16	1.56

According to the results, it seems likely that acetone and ether are more suitable for the morphogenesis of the cell than the others. Propanol and butanol were meager in promotion of the morphogenesis.

Discussion

The plant growth hormone has been shown to occur in species of brown algae, red algae, green algae and diatoms (duBuy and Olson, 1937; van Overbeek, 1940a. 1940b; Thimann *et al.*, 1942; Jacobs, 1951). Sandan and Ogura (1957) mentioned that IAA in low concentrations promoted both the morphogenesis and the protoplasmic rotation in the cell of *Nitella*. In the present work, NAA and IBA in low concentrations also accelerated the morphogenesis of the cell. Kögl *et al.* (1934) reported NAA mainly accelerated the root formation in higher plant. In regard to this fact it is interesting that NAA took more positive effect for rhizoid formation than for shooting in *Nitella* cell. In the cases of NAA, IBA and all vitamins so far used the optimum concentration for shooting was different from the one for rhizoid formation as in the case of IAA (Sandan and Ogura, 1957).

Yamada and Sandan (unpublished) observed that the protoplasmic streaming in the isolated internodal cell of Characeae was promoted for a long time by application of dilute solutions of fat soluble substances. The result of the present experiments showed the acceleration of morphogenesis in the cell of *Nitella* by application of fat

soluble substances in low concentrations. We are now in a position to say that the promotion of morphogenesis in *Nitella* cell synchronizes with the acceleration of the protoplasmic rotation in it. To emphasize this point, it is necessary to call attention to the fact that IAA in low concentrations promoted both morphogenesis and protoplasmic rotation in the cell of *Nitella* at the same time (Sadan and Ogura, 1957).

A complete interpretation of the promotion of morphogenesis induced by application of fat soluble substances in low concentrations is problematical. However, it is probable that this promotion of morphogenesis is closely related to the changes in the permeability of protoplasm.

In the present observations, all vitamins took positive effect on the new development of the cell. From the roles of the vitamins as constituents of coenzymes or other biochemical functions of them thus far known, it seems plausible that the vitamins promoted morphogenesis in the cell of *Nitella* in the present work.

Summary

1. NAA, IBA, vitamin B₁, B₂, B₆, C and nicotinic amide in appropriate concentration promoted the morphogenesis of the isolated internodal cell of *Nitella flexilis*. The optimum concentration of these reagents for shooting of the cell was different from the one for rhizoid formation. NAA was more favourable for rhizoid formation than for shooting.

2. Fat soluble substances in low concentrations accelerated the morphogenesis of the cell. Above all, dilute solutions of acetone and of ether were suitable as a culture solution of the cell.

The author wishes to express his most cordial thanks to Prof. N. Kamiya of Osaka University for his kind direction and helpful criticism throughout this work and also to Prof. T. Nakamura for his valuable advice.

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- 10) Yamada, Y. and Sandan, T., unpublished data.

本 会 記 事

役 員 異 動

4 月から役員が交代して、次のようになりました。

幹 事

幹 事 長：門司正三

庶務幹事：清水 碩

会計幹事：佐藤正一

編集幹事：吉田精一，岩城英夫，佐藤満彦

編集委員

千葉保胤，芳賀 恣，原 寛，宝月欣二，神谷宜郎，木村晴一郎，北村四郎，小林義雄，門司正三，長尾昌之，小野記彦，太田行人，大槻虎男，下郡山正巳，田川 隆，高宮 篤，渡辺清彦，亙理俊次，八巻敏雄，湯浅 明。

支 部 通 信

北 海 道 支 部

12 月例会 (12 月 7 日，於北大・理) 伊藤浩司：野附岬の放牧と植生。松浦一：中国を行く。

2 月例会 (2 月 15 日，於北大・理) 和気和民：クロカビ生長期の核酸について。田沢伸雄：ベニヒバ属 (*Ptilota*) の雄性生殖器官について。

東 北 支 部

32 年度 (第 10 回) 支部大会 (8 月 10 日，於浅虫) 工藤照夫：植物における Feulgen 反応について。I. 植物の Feulgen negativity について。工藤照夫・佐藤進一：植物における Feulgen 反応について。II. Feulgen 反応と pH の影響について (予報)。樋口利雄：福島県の蘚類 (石灰岩上蘚)。藪 邦彦：水稻種子中の発芽抑制物質。相馬寛吉：第四紀堆積物の花粉分析。中沢信午：進化の第一段階について。田中 清：アカマツ花粉の発芽伸長，でんぷん粒消失および形成に対する窒化ナトリウムの影響について。伊倉伊三美：シダ類精子の生存力に対する中性塩および pH の影響。倉石 衍：八甲田山の野生酵母 (予報)。柴岡孝雄・小田健二：車輪藻の節間細胞の呼吸。和田俊司：稲鞘葉のインドール酢酸吸収について。

10 周年を祝賀して動物学会東北支部と共催で

次の行事が行われた。

10 周年記念特別講演。郡場 寛「植物学の回顧」。10 周年祝賀会および懇親会。

中 部 支 部

第 49 回例会 (11 月 9 日，於名大・教養・生) 熊沢正夫：カラスライドによるインド，パキスタン方面諸国旅行談。

第 50 回例会 (1 月 25 日，於名大・理・生) 柄崎脩一：電子顕微鏡による細胞の微細胞構造について。原田市太郎：ミスオオバコ属に類似の水草 *Bootia* 属について。

近 畿 支 部

32 年度第 1 回例会 11 月 24 日，於大阪市大・理工) 河原 晨・増田芳雄：水草の Na 滲出，特にオーキシン作用について。増田芳雄：エンバクの子葉鞘の伸長に対するインドール酢酸の RNase の関係。小清水卓二：正倉院御物材質 (植物質) の報告。田中長三郎：柑橘類 (*Citrus*) の植物地理学的試説。〔特別講演〕山本武彦：細菌酵素の生成・分泌について。望月 明：カナダにおける小麦事情。

同年度第 2 回例会 (3 月 23 日，於神戸大・理・生) 辻 英夫，浜田秀男：イネおよびエンバクの発芽に伴う芽生中の酸溶性燐酸エステル量の変化。〔特別講演〕浜田秀男。印度支那調査旅行から帰って。

九 州 支 部

第 47 回例会 (11 月 30 日，於九大・理) 芳賀恣・野田昭三：「種」ツルボの細胞遺伝学的構造。稲田朝次：色素の生体内合成について (綜合抄録)。

熊本例会 (11 月 16 日，於熊本大・理) 宮田逸夫：コケの胞子の発芽と葉の再生。森 通保：稲田と蘭田の藻類について。

48 回例会 (2 月 8 日，於九大・理) 窪田日出夫：水分関係からみた生活形の意義。吉井甫：ヨーロッパ視察旅行談。

なお同支部では動物学会，生態学会と合同で第 8 回支部大会を 5 月 24 日・25 日の両日九大・農学部および理学部で開催いたします。

Obituary Note

Kwan KORIBA



With the unexpected death on December 15, 1957, of Kwan Koriba, President of Hirosaki University and Professor Emeritus of Kyoto University, the Botanical Society of Japan has lost one of its most distinguished honorary members, who was affiliated with the Society ever since his boyhood. Born in Aomori in 1882, Koriba attended middle school in Hirosaki, where he came back as the grey-haired President. His family graveyard faces Mt. Hak-koda, on the slopes of which the great botanist was brought up.

Koriba was graduated in 1907 from Imperial University of Tokyo, and took his doctor's degree five years later from the same University. Koriba was a professor at Tohoku Imperial University in Sapporo from 1915 to 1920, and then

joined Kyoto Imperial University. Retiring from the latter in 1942 at retirement age, he was appointed director of the Singapore Botanic Gardens, which were then under Japanese occupation. After returning home in 1946, he devoted himself to reading and writing until 1954, when he was persuaded by the earnest request of Hirosaki University to become its President. He was a leading figure in his native province ever since, and continued to be active until a few hours before his death.

"Living" in the mountains, young Koriba became interested in morphological and ecological observations. At Imperial University of Tokyo, he paid much attention to physiology because he thought morphological and ecological facts were ultimately to be explained from the physiological standpoint. He began experiments to observe the effects of the environmental factors on the variable morphological characters. He made elaborate experiments and observations for his thesis with the idea of elucidating modifications in phyllotaxis of the spike of *Spiranthes*.

Koriba was fond of observing vegetations. He visited the United States of America and European countries in 1918-1920, the Ponapé Islands in 1921, Java in 1929, South

America in 1931, and the northern part of China in 1940.

Vegetations and their successions on the volcanoes, the high mountains and the sand dunes invited Koriba's special attention, which resulted in the production of a number of papers. *Taeniophyllum*, Podostemonaceae and fossil *Hydrodictyon* awaited treatment by a morphologist and ecologist. Koriba published his theory in and after 1927 that the morphogenesis, including the differentiation of the floral as well as the sexual organs, should be explained on the basis of the effects of substances such as phytohormones and vitamins. He began phytoclimatological study in 1931, and made extensive research on evaporation and transpiration from 1936 to 1942. In Singapore, where the climate is nearly constant throughout the year, he made observation of the periodicity in growth and leaf shedding of many kinds of trees.

Koriba wrote a book on the forms of plants in 1951. In this unequalled book he discussed the plant forms from the viewpoints of organization, metamorphosis and function. In 1952 he published a comprehensive work on plant physiology and ecology.

Koriba planned to write a book on evolution in the plant kingdom. It is said that his book on the forms of plants was written for the purpose of introducing the discussions on evolution. In an age of ever-increasing specialization, only a few are qualified to synthesize diverging fields to explain phenomena of life on sound ground. Such a man was Koriba. In his notes left behind we find that he intended to discuss evolution by synthesizing the morphological, physiological, ecological and genetical points of view. He was really a great biologist.

Koriba was a beloved son of Mt. Hakkoda. He behaved, loved and hated as a man of Nature. Even under the military rule he was on the side of Nature and science, as we find him described in the post-War Gardens' Bulletin, Singapore, 1947. Those who were privileged to know him will certainly keep long the inspiration imparted by him. A life devoted not only to science but also to education in the true sense of the word.

Joji ASHIDA

Studies on Cerophilic Growth of Moulds on Wax and Paraffin. II.

Comparative Studies on Cerophilic and Tonophilic Growth of Moulds.

by Morieko IMAI*

今井百里江子*: 糸状菌の蠟およびパラフィン上の発育について. II.
好蠟的発育と好肥的発育の比較.

Received September 30, 1957

Introduction

In one of the previous papers¹⁾ of this series of investigations, the author has described a new species of fungus, *Acrotheca cerophila*.²⁾ The organism was originally isolated from a growth covering the surface of the waxy substance on the nodes of bamboo culms and it was actually shown to be cerophilic in the nature of its nutritive requirements, being capable of growing on synthetic media which contained wax or paraffin as the sole source of carbon. In view of the fact that its natural habitat was the kind described above, it was suspected that the organism might be a tonophilic form. The problem seemed to us to have a certain interest in connection with investigations on the tonophilic growth of moulds which have been carried on for several years in this laboratory^{3), 4), 5), 6)}. For the purpose of comparison, parallel experiments were carried out with several representative species of ordinary moulds as well as the typically tonophilic form mentioned above. In the following, the results of the experiments will be briefly described.

Methods and results

(A) *Cultures on hypertonic growth media.* For the purpose of obtaining various grades of physiological dryness, varied amounts of sodium chloride (0, 9.1, 13.6, and 22.2% NaCl) were added to the basal medium (Czapek's solution), and 2 to 5% agar. The reaction of the medium was found to be about pH 5.6-5.8, as measured after sterilization in an autoclave. The agar slants were inoculated with spores of the moulds and incubated at 30° for periods of two weeks or longer. The extent of growth is represented by the number of crosses in Table 1; (≡) indicates most vigorous growth of mycelium covering the whole surface of the agar; (±) stands for scanty growth hardly exceeding the stage of spore germination; (—) indicates "no growth" as determined by minute examination with a magnifying glass.

*Botanical Laboratory, Faculty of Science, Ochanomizu University, Tokyo, Japan. お茶の水女子大学理学部植物学教室

Table 1. Cultures on hypertonic media.*

Organism	Incubation time (days)	Concentration of NaCl (%)				
		0	9.1	13.6	16.6	22.2
<i>Aspergillus glaucus</i> var. <i>tonophilus</i> †	4	—	±	+	—	—
	8	—	±	++	+	—
	10	—	±	++	++	—
	17	—	##	##	##	—
<i>Aspergillus niger</i> †††	4	##	##	—	±	—
	8	##	##	++	##	—
	10	##	##	##	##	—
	17	##	##	##	##	—
<i>Penicillium notatum</i> P 1††	4	##	##	++	++	—
	8	##	##	##	++	—
	10	##	##	##	##	—
	17	##	##	##	##	—
<i>Penicillium notatum</i> O 6††	4	##	##	++	##	—
	8	##	##	##	##	—
	10	##	##	##	##	—
	17	##	##	##	##	—
<i>Penicillium</i> sp. PX††	4	##	++	++	++	—
	8	##	##	##	##	—
	10	##	##	##	##	—
	17	##	##	##	##	—
<i>Acrotheca cerophila</i> †††	4	##	—	—	—	—
	8	##	##	++	+	—
	10	##	##	++	++	—
	17	##	##	##	++	—
<i>Aspergillus flavus</i> †††	4	##	+	++	+	—
	8	##	##	##	+	—
	10	##	##	##	++	—
	17	##	##	##	##	—
<i>Aspergillus oryzae</i> ††	4	##	##	++	—	—
	8	##	##	##	+	—
	10	##	##	##	++	—
	17	##	##	##	##	—
<i>Aspergillus oryzae</i> 383††	4	##	##	++	+	—
	8	##	##	++	+	—
	10	##	##	##	++	—
	17	##	##	##	##	—
<i>Aspergillus</i> <i>fumigatus</i> 5052†††	4	##	—	—	—	—
	8	##	##	++	—	—
	10	##	##	++	+	—
	17	##	##	##	+	—
<i>Aspergillus</i> <i>fumigatus</i> 5442†††	4	##	—	+	+	—
	8	##	##	++	+	—
	10	##	##	##	++	—
	17	##	##	##	##	—
<i>Aspergillus</i> <i>fumigatus</i> 5445†††	4	##	—	—	—	—
	8	##	##	+	—	—
	10	##	##	++	—	—
	17	##	##	##	+	—

<i>Monascus araneosus</i> †††	4	—	—	—	—	—
	8		—	—	—	—
	10		—	—	—	—
	17		+	+	—	—
<i>Cladosporium herbarum</i> ††	4		—	—	—	—
	8		—	—	—	—
	10		+	+	—	—
	17		+		—	—
<i>Alternaria</i> sp.†	4			—	—	—
	8			—	—	—
	10			+	—	—
	17 ^a			+	+	—
<i>Curvularia</i> sp. A 2†	4		+	+	—	—
	8			+	—	—
	10			+	—	—
	17				+	—

††† (cerophilic), †† (less marked cerophilic), † (non-cerophilic): Grade of cerophily.
* Composition of culture media, see text.

The experimental results are shown in Table 1. If we use “halophily” to define the response of the test organism towards higher concentrations of salt in the growth medium, the moulds in the above experiments will be divided into the following three groups;

1) Stenohalophilic form, showing favorable growth only in the presence of higher concentrations of salt in the growth medium;

Aspergillus glaucus var. *tonophilus*.

2) Euryhalophilic forms, showing favorable growth over a wide range of salt concentrations;

Aspergillus niger, *Penicillium notatum* P 1, *Penicillium notatum* O 6, *Penicillium* sp. PX, *Acrotheca cerophila*, *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus oryzae* 383, *Aspergillus fumigatus* 5052, *Aspergillus fumigatus* 5442, and *Aspergillus fumigatus* 5454.

(3) Non-halophilic forms, showing no growth or scanty growth above certain, relatively dilute, salt concentrations;

Monascus araneosus, *Cladosporium herbarum*, *Alternaria* sp., *Curvularia* sp. A 2.

There is, however, a gradual transfer from one group to another, which obscures the boundaries of these groupings. Indeed, with the lower members of the euryhalophilic group in the above list, the author is not very sure whether they merit the term “tonophilic”. The demarcation between “positive growth” and “no growth” is much more distinct in the case of the third group of organisms in the above classification. The author is inclined to denote these organisms as “salt sensitive” rather than to the more modest expression, “non-halophilic”. This seems to be fully demonstrated in the case of *Monascus araneosus* and *Cladosporium herbarum* (see Table 1).

B) *Cultures under varied atmospheric humidity.* Spores were inoculated on the underside of a slide glass mounted on the top of a small glass tube (20 mm. in diameter; 30 mm. in height), half filled with aqueous solution of sulfuric acid of

varied concentrations to maintain the atmosphere inside the tube at the desired level of relative humidity. The tubes were incubated at 30° and were subjected to continual microscopic observation with respect to germination of the spores and their subsequent growth.

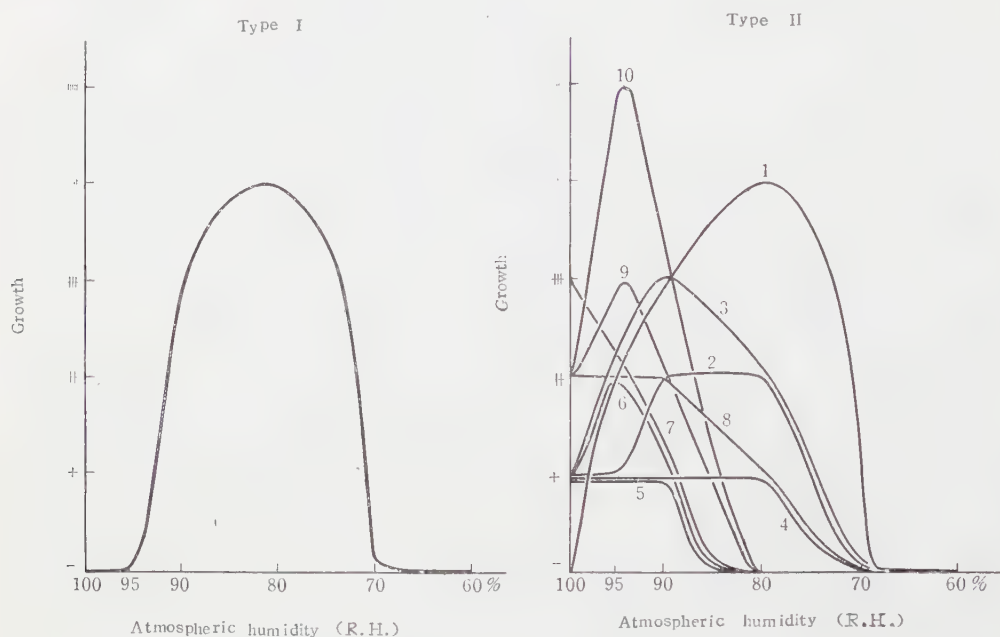


Fig.1. Germination and growth of moulds as influenced by atmospheric humidity (R.H.).

Type I. 1. *Aspergillus glaucus* var. *tonophilus*

Type II. 1. *Aspergillus niger*

2. *Penicillium notatum* P1

3. *Aspergillus flavus*

4. *Aspergillus oryzae* 383 and *Aspergillus fumigatus* 5052

5. *Aspergillus fumigatus* 5442

6. *Curvularia* sp. A2

7. *Aspergillus fumigatus* 5454

8. *Aspergillus oryzae*

9. *Monascus araneosus*

10. *Acrotheca cerophila*

The results are presented in Figure 1. Here also, growth is denoted in terms of an arbitrarily defined extent of microbial development; the crosses from (###) to (+) indicate more or less marked growth of hyphae from the spores inoculated on the glass surface; a greater number of crosses expressing more enhanced growth observed; (—) shows that there was no indication of spore germination as examined from time to time during an incubation period as long as 60 days.

The most prominent feature of the experimental results is the characteristic behaviour of *Aspergillus glaucus* var. *tonophilus*, which showed optimal growth at a relatively lower level of atmospheric humidity (about 80% R.H.). The growth of

this organism was actually found to be suppressed at 100% R.H., no germination of spores taking place under this incubation condition. An almost similar behaviour towards humidity was shown by another common mould, *Aspergillus niger*, except this mould could germinate at 100% R.H. (see Fig. 1). Other mould species tested in the present study showed all types of response in this respect, from the versatile type represented by *Penicillium notatum* P1 to the less adaptable type including most other forms such as *Monascus araneosus*, *Curvularia* sp. A2, *Aspergillus fumigatus* 5442 and *Aspergillus fumigatus* 5454. The growth of the last-mentioned group of moulds is indeed restricted to the highest level of atmospheric humidity, above 90%, so far as the experiments in this study are concerned. It was rather disappointing to the author to find that the cerophilic organism, *Acrotheca cerophila*, presented no particularly characteristic behaviour in this respect. Indeed, *Monascus araneosus* and *Cladosporium herbarum*, which have been classified by the author under the non-halophilic (salt-sensitive) group belonged to the moisture-loving group in the experiments under discussion, their optimum atmospheric humidity for growth lying as high as 95% or more. The results of this set of experiments are summarized in Table 2, together with those described in the previous section. The term "xerophily" is meant here to denote the favorable response of the test organism towards atmospheric dryness in the cover glass germination test described above.

It will be deduced from the table that there is a close relationship between the two expressions of the response of the test organisms toward the water content of

Table 2. Cerophilic and tonophilic nature of moulds.

Organism	Tonophily		Cerophily
	Xerophily	Halophily	
<i>Aspergillus glaucus</i> var. <i>tonophilus</i>	stenoxerophilic	stenohalophilic	C (−)
<i>Aspergillus niger</i>	euryxerophilic	euryhalophilic	C (+)
<i>Penicillium</i> sp. PX	—	"	C (±)
<i>Penicillium notatum</i> P1	"	"	C (±)
<i>Penicillium notatum</i> O6	—	"	C (±)
<i>Aspergillus flavus</i>	"	"	C (+)
<i>Aspergillus oryzae</i>	"	"	C (±)
<i>Aspergillus oryzae</i> 383	"	"	C (±)
<i>Aspergillus fumigatus</i> 5052	"	"	C (+)
<i>Aspergillus fumigatus</i> 5442	"	"	C (+)
<i>Aspergillus fumigatus</i> 5454	"	"	C (+)
<i>Acrotheca cerophila</i>	"	"	C (+)
<i>Alternaria</i> sp.	—	non-halophilic	C (−)
<i>Curvularia</i> sp. AZ	"	"	C (−)
<i>Cladosporium herbarum</i>	—	"	C (±)
<i>Monascus araneosus</i>	—	"	C (+)

Grade of cerophily: C(+) cerophilic; C(±) less marked cerophilic; C(−) non-cerophilic.

their environment: i.e., “xerophily” on the one hand, and “halophily” on the other.

C) *Tonophily as influenced by cerophilic mode of growth.* Another series of experiments was planned to investigate whether the tonophilic nature of a mould depends on its nutritional condition. The growth on a sucrose medium was compared with that on a paraffin medium, with and without the addition of sodium chloride as the osmoactive substance. The composition of each culture medium was as follows: Ordinary sucrose medium: 3% sucrose added to Czapek’s agar; hypertonic sucrose medium: 16.6% sodium chloride added to the above. Ordinary paraffin medium: 0.7 g. paraffin (Merck; M.P. 55~56°) for each test tube containing 12 ml. of basal agar. Hypertonic paraffin medium: 16.6% sodium chloride added to the above.¹⁰

The pH of the above media determined after sterilization in an autoclave was found to be 5.6~5.8. The slants inoculated with spores were incubated at 30° and the growth was followed until the final stationary state was reached.

The results of the experiments are presented in Table 3. In accordance with the results reported in a foregoing paper¹⁾ the growth on ordinary sucrose medium was most vigorous, except in the case of the strictly tonophilic organism, *Aspergillus glaucus* var. *tonophilus*. The growth on hypertonic sucrose medium was¹¹

Table 3. Cerophilic growth of moulds as influenced by tonocity of culture medium.

Medium*		Usual medium				Hypertonic medium			
Carbon source		Sucrose		Paraffin		Sucrose		Paraffin	
Organism	Maximum growth	Growth	Incubation time (days)	Growth	Incubation time (days)	Growth	Incubation time (days)	Growth	Incubation time (days)
<i>Aspergillus glaucus</i> var. <i>tonophilus</i>		—	63	—	63	+++	13~31	—	63
<i>Aspergillus oryzae</i>		+++	3	±	10	+++	20	—	“
<i>Aspergillus oryzae</i> 383		+++	3	±	13	+++	23	—	“
<i>Aspergillus niger</i>		+++	3	+++~+++	28	+++	7	—	“
<i>Penicillium notatum</i> F1		+++	3	+	10	+++	9	—	“
<i>Penicillium notatum</i> O6		+++	3	+	10	+++	7	—	“
<i>Penicillium</i> sp. PX		+++	5	++	36	+++	9	—	“
<i>Acrotheca cerophila</i>		+++	5—7	+++	15	++	19~63	—	“
<i>Aspergillus flavus</i>		+++	3	+++	15	++~+++	13~20	—	“
<i>Aspergillus fumigatus</i> 5052		+++	4	+++	28	±	63	—	“
<i>Aspergillus fumigatus</i> 5442		+++	3	+++	20	+	63	—	“
<i>Aspergillus fumigatus</i> 5454		+++	4	+++	28	+~++	63	—	“
<i>Alternaria</i> sp.		+++	4	—	63	—~±	63	—	“
<i>Curvularia</i> sp. A2		+++	4	—	63	—~±	63	—	“
<i>Monascus araneosus</i>		+++	5	+++	36	—	63	—	“
<i>Cladosporium herbarum</i>		+++	3	±	15	—	63	—	“

* Composition of culture media, see text.

also luxuriant in the last-named form and other facultative-tonophilic forms including *Aspergillus oryzae*, *Aspergillus oryzae* 383, *Aspergillus niger*, *Penicillium notatum* P1, *Penicillium notatum* O6, and *Penicillium* sp. PX, although there was more or less marked retardation of growth caused by high concentration of salt, as indicated by the increase in length of culture period necessary for the full development of the mould to take place (see Table 3).

Marked inhibition under hypertonicity was observed in the case of less halophilic forms such as *Acrotheca cerophila*, *Aspergillus flavus*, *Aspergillus fumigatus* 5052, *Aspergillus fumigatus* 5442, *Aspergillus fumigatus* 5454, *Alternaria* sp. and *Curvularia* sp. A2; no growth whatsoever took place in *Monascus araneosus* and *Cladosporium herbarum*. With cultures on paraffin, it was most remarkable that cerophilic growth was strongly affected by high tonicity of the medium, no growth ever taking place on hypertonic medium, even with those typically cerophilic forms such as *Acrotheca cerophila*, *Aspergillus flavus*, *Aspergillus fumigatus* 5052, *Aspergillus fumigatus* 5442, *Aspergillus fumigatus* 5454 *Aspergillus niger*, and *Monascus araneosus*.

The inhibitory effect of high tonicity (or high salt concentration) on the ordinary growth (with sucrose) and on cerophilic growth (with paraffin) was investigated in more detail, using *Acrotheca cerophila* and *Aspergillus niger* as the test organisms (Table 4). It is again clearly shown that the cerophilic growth of these moulds is more strongly affected by high tonicity than its growth on sucrose, even in the case of tonophilic organism.

Table 4. Development of mould growth on sucrose and paraffin as influenced by tonicity of culture medium.*

Organism	<i>Acrotheca cerophila</i>								<i>Aspergillus niger</i>							
Carbon source	Sucrose				Paraffin				Sucrose				Paraffin			
NaCl concentration (%)	0	5.9	12.2	18	0	5.9	12.2	18	0	5.9	12.2	18	0	5.9	12.2	18
Incubation time (days)																
3	+	±	—	—	—	—	—	—	+++	+++	+	—	—	—	—	—
5	+++	+	±	—	+	—	—	—	+++	+++	++	—	+	—	—	—
7	+++	+++	+	—	++	—	—	—	+++	+++	+++	—	+	+	—	—
9	+++	+++	+	—	++	+	—	—	+++	+++	+++	—	+	+	—	—
14	+++	+++	+	—	+++	+	—	—	+++	+++	+++	—	++	++	—	—
18	+++	+++	+	—	+++	+++	—	—	+++	+++	+++	—	++	++	—	—
24	+++	+++	+	—	+++	+++	—	—	+++	+++	+++	—	++	++	—	—

* Composition of culture media, see text.

On the basis of these experimental results, it seems most likely that there is no direct relationship between “cerophily” and “tonophily” in moulds.

The author wishes to express her hearty thanks to Prof. T. Ohtsuki, Prof. Y.

Miyamoto, and Prof. A. Takamiya for their valuable advice throughout the progress of this study, and also to Dr. K. Tsubaki for his courtesy in giving these above mentioned mould strains.

Summary

Sixteen strains of moulds were examined on the point of cerophilic growth and tonophilic.

(1) Cultures were performed on hypertonic growth media containing varied concentrations of sodium chloride. The tested mould strains were divided into three groups, namely "steno-", "eury-" and "non-halophilic" forms.

(2) Cultures were performed under varied atmospheric humidity. The results obtained are shown in Figure 1 and the results of this set of experiments are summarized in Table 2, together with those described in the previous section.

(3) In order to elucidate whether the tonophilic nature of mould depends on its nutritional condition, another series of experiments was performed. The results obtained were shown in Table 3 and 4.

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On the Application of ^{14}C -Method to Measuring Organic Matter Production in the Lake

by Shun-ei ICHIMURA* and Yatsuka SAIJŌ**

市村俊英*・西条八重**：湖沼における物質生産測定への ^{14}C -法の応用について

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Since Steemann Nielsen (1952) used the ^{14}C technique on the Galathea expedition, the ^{14}C -method has been known as the most sensitive technique for measuring primary production in waters. However, many pending questions still remain in this method, though the critical test has been made by several investigators (Steemann Nielsen 1952, 1957, Ryther and Vaccaro 1954, Ryther 1956, 1957, Miyake et al. 1954, Doty 1956,

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan. 東京教育大学理学部植物学教室

** Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Setagaya, Tokyo, Japan. 東京都立大学理学部化学教室

1957). Moreover, almost all of these studies were concerned with the sea water and ¹⁴C has not yet been employed adequately in lakes. It seems to be indispensable to scrutinize the fitness of this method to measurement of the primary production of lakes.

In the present paper the authors will discuss the application of the ¹⁴C-method to the fresh water in comparison with the well known O₂-method.

Methods

The method used in this paper was similar in principle to Steemann Nielsen's (1952) and Ryther's (1954). Briefly stated, natural water or suspension of pure culture of algae was filled in a clear and a dark glass bottle and then added with 1 ml. of radioactive sodium carbonate solution containing 1 or 4 μc. of ¹⁴C. After exposure for about 3 to 6 hours, or in some cases for 24 to 48 hours, the phytoplankton in the bottle was filtrated with a membrane filter, then washed and dried, and its radioactivity was measured with a G.M. counter. The absolute CO₂ assimilation of phytoplankton was calculated as follows;

CO₂ assim.=Total CO₂ in water × $\frac{\text{Activity of } ^{14}\text{C assim. in phytoplankton}}{\text{Activity of } ^{14}\text{C added in water}}$

Along with ¹⁴C-method, O₂-method was used for determining assimilation and respiration of phytoplankton. Each result was verified with double or triple observations. The amount of total CO₂ in water was measured by Saijō's method (1956), by which even

Table 1. Total CO₂ concentration in surface water of some lakes

Lake type	Lake	Date	Total CO ₂ mg./l.
Oligotrophic	Akagi-onuma	July 26, 1955	8.4
	Biwako	Sept. 15, 1955	18.3
	Ashinoko	June 22, 1957	20.0
Mesotrophic	Harunako	Sept. 29, 1957	12.6
	Shinseiko	July 13, 1955	25.9
Eutrophic	Kasumigaura	Sept. 16, 1956	26.6
	Teganuma	March 26, 1957	34.7
	Nakanuma	May 30, 1957	42.0
	Jōnuma	Oct. 27, 1956	71.0

low content of CO₂ was measured in field with an accuracy of 1.5 % only within 15 minutes. The values thus gained in some Japanese lakes are summarized in Table 1. Phytoplankton density was determined by pigment analysis and was indicated as chlorophyll amount. In all cases, O₂ equivalent of CO₂ assimilation was calculated under assumption of R.Q.=1, without consideration on the 6% depression of ¹⁴C assimilation in comparison with ¹²C-assimilation (Steemann Nielsen 1952). Fluctuation of some of results gained with ¹⁴C-method is summarized in Table 2, It seems,

Table 2. Fluctuation of count number measured in each sample taken from some waters

Material	Nakanuma raw water		Teganuma raw water		<i>Chlorella</i> sp. pure cult.
Duration hr.	14		2		2
Condition	Laboratory 20°		Laboratory 22°		Laboratory 20°
	12 Klux	Dark	12 Klux	Dark	12 Klux
Assimilation as count/min.	126	6	139	4	329
	128	9	141 ^{1/}		352
	131		145		368
	135		156		376
	162				

however, to be mainly caused by heterogeneous distribution of phytoplankton in the suspension rather than by technical error.

Results and Discussion

1. Relation of the photosynthesis to the light intensity

Pure culture of *Chlorella* sp. was suspended in 1/5 concentration of Detmer's solution and the suspensions were exposed to various light intensities at 22° in 100 ml. glass bottles. As light source, a 500 W flood lamp was used. The initial total CO₂ in the suspension was 20 mg./l. and the chlorophyll concentration was 0.052 mg./l. Similar values were observed in natural eutrophic lakes in Japan (Ichimura 1956). Photosynthesis was measured after 2 hour exposure to light, during which the bottles were rotated at twenty minute intervals in order to prevent the algae from sedimentation.

Between the photosynthetic rates which were determined with ¹⁴C-and O₂-methods good agreement was proved in higher illumination (Fig. 1), though in lower illumination there was a discrepancy which

might be caused by CO₂ dark fixation. This result coincides fairly well with Ryther's (1956).

The influence of CO₂ concentration on photosynthesis was investigated with both the methods. Various amount of sodium bicarbonate was added into 1/5 Detmer's solution. The concentration of total CO₂ was 5.5, 11, 22, and 44 mg./l., respectively; these fall in the range of natural total CO₂ concentration in Japanese lakes. Fig. 2 shows the results obtained at 20° and in 12 kilolux illumination. The CO₂ saturation in photosynthesis

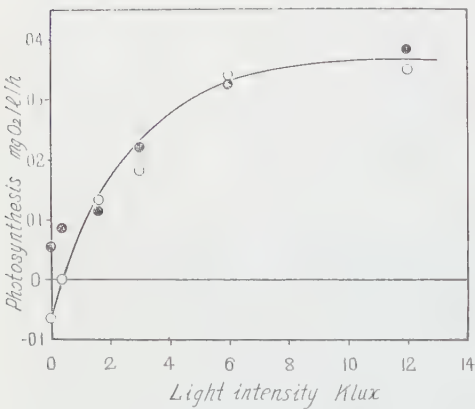


Fig. 1. Photosynthesis measured by O₂(○) and ¹⁴C(●) methods in a pure culture of *Chlorella* sp.

occurred at about 5 mg. CO₂ per litre, afterwards increase of CO₂ concentration caused only slight increase of photosynthesis. No special difference was detectable between the values obtained with ¹⁴C- and O₂-methods.

Further comparison of both methods was carried out on raw water sampled from 1 m. depth of lakes. The experiments were started under laboratory condition within 2-6 hours after sampling. Exposure time was varied depending on the phytoplankton concentration; it was usually about 2 hours for eutrophic lakes samples and 6 hours for oligotrophic ones. Fig. 3 indicates the light assimilation curves obtained with the samples collected from Lake Teganuma (eutrophic) and Lake Haruna (mesotrophic). Dominant phytoplankters in the former were *Fragillaria crotonensis* and *Tabel-laria fenestrata*, and in the latter they were *Melosira italica*, *Eudorina elegance*, etc. A fairly close agreement with the result obtained in *Chlorella* suspension was found in the case of Lake Teganuma. The data at Lake Haruna also showed similar trend, though it is not so clear as at Lake Teganuma. In Table 3 the main features obtained with both methods are summarized.

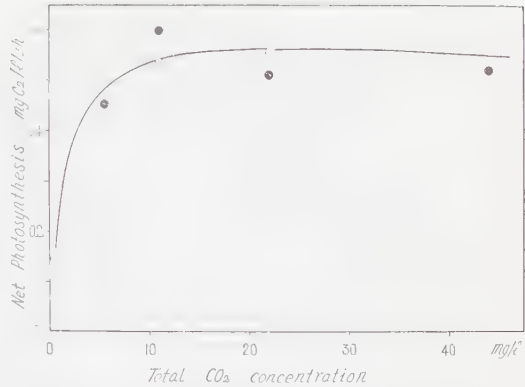


Fig. 2. Relation between photosynthesis and total CO₂ concentration measured by O₂ (○)- and ¹⁴C (●)-methods in a pure culture of *Chlorella* sp.

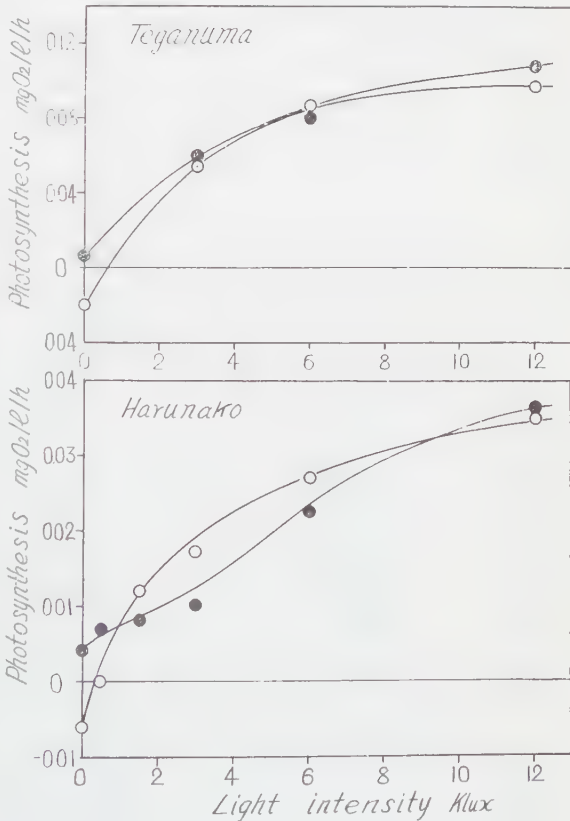


Fig. 3. Photosynthesis measured by O₂ (○)- and ¹⁴C (●)-method in the sample waters taken from Lake Teganuma (eutrophic) and Lake Harunako (mesotrophic).

Table 3. Main photosynthetic features obtained by ¹⁴C- and O₂-methods in sample waters taken from Lake Teganuma and Lake Harunako. Figures in brackets denote mg. oxygen per mg. chlorophyll per hour

Lake	Total CO ₂ mg./l.	¹⁴ C-method mg. O ₂ /l./hr.		O ₂ -method mg. O ₂ /l./hr.	
		Light	Light-Dark	Gross.	Net.
Teganuma	34.7	0.112 (6.6)	0.106 (6.2)	0.123 (7.2)	0.099 (5.8)
Harunako	12.6	0.036 (4.8)	0.031 (4.1)	0.041 (5.5)	0.035 (4.7)

2. Determination of photosynthetic rate under field condition.

The general procedure was similar to that described previously. Photosynthesis was measured with so-called "in situ" method. Namely water samples taken up from various depths of lakes were suspended, after filling the 100 ml. or 200 ml. clear and dark bottles, to the respective depths where they had been sampled. In parallel with this, light intensity, temperature, chlorophyll and nutrient salt contents (PO₄-P, NH₄-N, NO₃-N) in the water were determined by usual methods.

The photosynthetic capacity of water alters with light intensity, and the density of phytoplankton varies with water depth. Fig. 4-A shows the vertical change of the

photosynthetic capacity of water at Lake Nakanuma, which was determined at the deepest part of the lake in May. The bottles were kept in water for two days (a cloudy and a rainy). In the surface layer the value determined with ¹⁴C-method agreed closely with the net assimilation measured with O₂-method, but in low light intensity at or below the compensation depth, the former value was greater than that of O₂-method as expected from the results mentioned already. Fig. 4-B indicates the results obtained at Lake Kasumigaura on a cloudy day of November 1956. Samples were suspended in the lake from noon to next noon. Prevailing dominant phytoplanktoner was *Microsistis* sp. In case of measuring productivity under field condition, besides the photosynthesis itself, dark fixation of CO₂ should be taken into consideration. Intensity of dark fixation in lake water, so far as we know, was usually only few per cent of that of photosynthesis under optimal light condition, and it seems that the dark fixation is generally not so im-

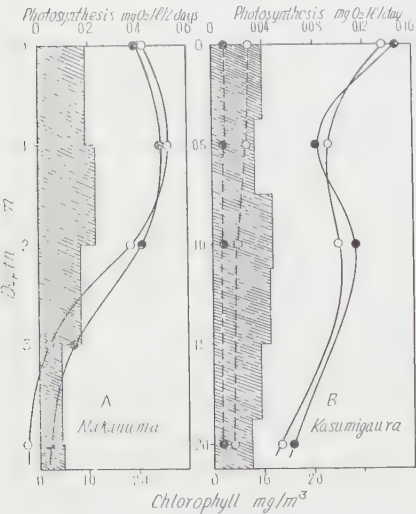


Fig. 3. Vertical changes of photosynthetic rate measured by O₂- and ¹⁴C-methods in Lake Nakanuma and Lake Kasumigaura.

O₂-method (—○—, net-photosynthesis; ---●---, respiration) ¹⁴C-method (●, photosynthesis; ---●---, dark fixation)

portant in the primary production of the lake as for that of the sea. However, occasionally high values of dark fixation have also been measured in the authors experiments. It is therefore desirable to determine CO_2 dark fixation in each case in parallel with the measurement of photosynthesis. The authors will treat this problem in other paper in detail.

3. Relation of duration time to the photosynthetic rate

It has been said that phytoplankton enclosed in a bottle cannot keep its normal photosynthetic activity under the unnatural condition for a long time. As the duration time, 2-6 hours are generally adopted (Verduin 1954). Effect of long duration on photosynthetic rate should be examined with the ^{14}C -method, too. Fig. 5-A shows the change of photosynthetic rate with time course, in continuous illumination of 12 kilolux at 22° . Water was sampled from Lake Nakanuma in April 1956. During the first six hours, photosynthesis proceeded actively and lineary correlated with the time elapsed, but afterwards activity declined gradually. Same trend was also observed in an experiment with sea water (Fig. 5-B). The dark fixation of CO_2 was extremely low in both the cases. Similar experiment was done at Lake Ashinoko (oligotrophic) with the "in

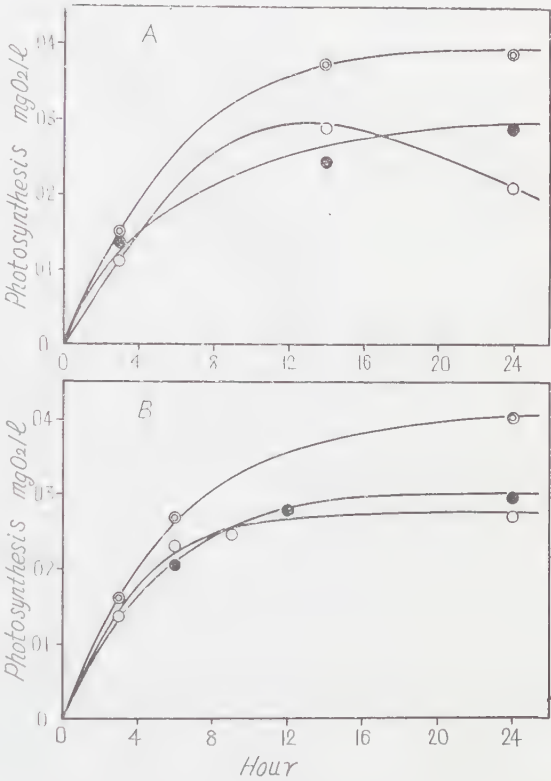


Fig. 5. Effect of duration time on photosynthesis in continuous illumination measured by O_2 and ^{14}C methods in natural waters. O_2 method (○-gross-photosynthesis: ● net-photosynthesis). ^{14}C -method (● photosynthesis)

Table 4. Relation of duration time to photosynthetic rate obtained by O_2 - and ^{14}C -methods in Lake Ashinoko in July. First two days were rainy and last day was fine

Duration time	^{14}C -method mg. O_2 /l.		O_2 -method mg. O_2 /l.		
	Light	Dark	Net	Resp.	Gross
6 hours (noon to sunset)	0.007	0.002	0.008	0.002	0.010
24 " (noon to next noon)	0.017	0.003	0.017	0.005	0.022
48 " "	0.028	—	0.037	—	—

situ" method on a cloudy day of July. The sample water was collected from 1 m. depth and some bottles were suspended at the same depth from noon to sunset, and the others were suspended from noon to next noon. The results are summarized in Table 4. The total photosynthesis throughout a day was almost the double of a half day value.

Concerning this, other experiment was carried out with O_2 -method at Lake Kasumigaura in June. Photosynthesis during 12 hours was measured in two ways, i.e. one was summing of the photosynthesis measured every two hours, and the other was determining photosynthesis at sunset after a continuous 12 hour exposure. It was sunny during the experiments. Real assimilation of 0.85 mg. O_2 /l. was calculated in the former and 0.67 mg. O_2 /l. was obtained in the latter. The authors confirmed that the duration of exposure should not exceed half a day, because the continuous exposure from sunrise to sunset gave a depressed productivity. However, when a longer exposure is inevitable, it is recommended that the experiment will be started at noon and finished at the next noon.

Summary

In this paper the authors tried some fundamental investigations on the ^{14}C -method for measuring primary production in the lake in comparison with the O_2 -method.

From the results obtained it was confirmed that the ^{14}C -method is useful for the measurement of the net photosynthesis and the exposure to light for the measurement should not exceed half a day even though in case of the ^{14}C -method.

In general, dark fixation of CO_2 in lake is low, but occasionally high abnormal values were measured in some waters. It is therefore desirable to determine the dark fixation simultaneously along with the photosynthesis.

Since the ^{14}C -method is not fit for measuring respiration, proper allowances for respiration should be made in order to calculate the gross photosynthesis.

The authors wish to express their cordial gratitude to Prof. M. Monsi and Prof. K. Hogeitsu who gave the authors helpful suggestions and valuable advice. The authors also desire to express their thanks to Prof. K. Noguchi and Prof. T. Hanya for their kind support for the present research.

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On the Relationship between Plant Growth and Nitrogen Supply under Water Culture Conditions

Relations of Plant Communities to Edaphic Factors with
Special Reference to Mineral Nutrition I.

by Yasuhiko TEZUKA*

手塚泰彦*: 水耕条件での植物の生長と窒素供給量との関係・
無機栄養からみた植物群落と土壌条件の関係 I.

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Since mineral nutrients are, together with light, water, carbon dioxide etc., essential for plant growth, their ecological effects on plant communities have been studied for many years^{8), 16)}. Recently, Billings⁽¹⁾ has reviewed many investigations in this field. However, the effect of soil chemical properties on plant communities seems to have been explained on the rather weak basis of observations or assumptions. Therefore, some experimental studies are expected for the better understanding of mineral nutrient economy of a plant, or of a plant community. The mineral nutrient economy interacts so closely with dry matter production of the plant community, which is rather directly dependent on its productive structure, that the studies of dry matter production and productive structure have to be pursued simultaneously for the sake of proper evaluation of the mineral nutrient economy.

Concerning this, the author studied with water culture of *Chrysanthemum coronarium* var. *spatiosum* the influence of nitrogen ion concentration upon the ion absorption and plant growth in connection with dry matter production; change of nitrogen concentration in plant tissue; and the effect of nitrogen deficiency on the concentration of other mineral elements in tissue.

Material and Method

As material *Chrysanthemum coronarium* var. *spatiosum* was used on account of small seeds and rapid reaction to nutrient conditions. After ten days from germination, 37 seedlings whose mean dry weight was 1 mg. were transplanted to a water culture pot being 15 cm. in diameter and each containing 2 liters of nutrient solution. One liter of the standard nutrient solution modified from Boysen Jensen's³⁾ had 1.00 g. KNO₃, 0.25 g. CaCl₂·6H₂O, 0.25 g. MgSO₄·7H₂O, 0.05 g. KH₂PO₄, 5 ml. of 0.8% Fe-citrate, and 1.5 ml. micronutrient solution, whose constitution was the same as Boysen Jensen's. The pH of the solution was 5.8.

Four nitrogen levels, 0, 5, 20 and 100 p.p.m. of nitrate nitrogen, were prepared by change of KNO₃ amount in the standard solution, compensating the potassium con-

* Department of Biology, Faculty of Science, Tokyo Metropolitan University, Tokyo, Japan.
東京都立大学理学部生物学教室.

centration with addition of KCl. The culture solutions were not renewed throughout the growth period, for a preliminary experiment showed no marked difference in the initial growth under the conditions of 1 and 10 p.p.m. nitrogen levels. The water level in the pots was kept at 2 cm. below the lid supporting the plants, frequently adding distilled water, and it prevented detectable deficiency of oxygen in the solutions. The pots were in a frame outdoors for 50 days, from July 15, 1954. Five plants per pot, of 4 or 5 series, were sampled at random on every 10th day.

Chlorophyll content of leaves was determined photoelectrically, after converting it to pheophytin. Microkjeldahl method was adopted for measurement of total nitrogen in plant material, and for determination of nitrate nitrogen residue in the culture solutions phenoldisulfonic acid method was used. Total phosphorus, calcium, and iron were determined with ammonium-molybdate method (according to Denigè), Ca-oxalate method, and dipyriddy method, respectively.

Photosynthesis, and respiration of leaves and stems were measured at excised organs with a modified Boysen Jensen's method (1932). For the measurement of respiration of roots (excised) Winkler's method was adopted.

Results and Discussion

a) **Amount of nitrogen and plant growth:**— Relations between the amount of

nitrogen supply and plant growth are shown in Fig. 1, where it is clear that the maximal weights of the plants were approximately proportional to the total amount of nitrogen supplied, although all the growth curves ran in sigmoid shape with same initial growth rate until they reached a limitation given by nitrogen deficiency in the nutrient solution. Therefore, the initial growth rate seems to be independent of nitrogen concentrations in the range of the experiment. This differs from the results obtained by e. g. Hoagland *et al*⁵⁾ and Overstreet *et al*¹⁵⁾.

In order to assure the fact, another water culture experiment was carried out in the autumn 1955. Two pots of 15.5 p.p.m. and 16.2 p.p.m. of initial nitrate nitrogen concentrations were prepared, besides a control pot of

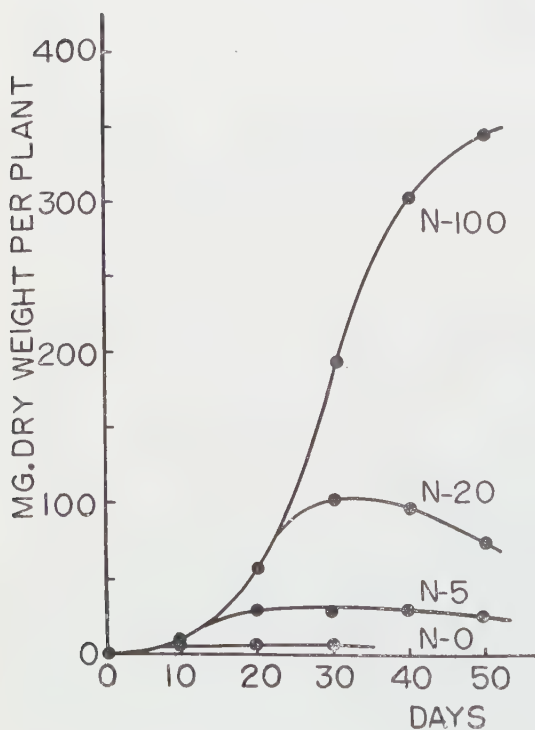


Fig. 1. Growth curves under various nitrogen supply. See text.

150 p.p.m. nitrate nitrogen. The procedure was the same as above experiment. It can be seen in Fig. 2 that nitrogen concentration in the solutions diminished linearly with approximately linear increase of dry weight of the plants, and there was no difference in the dry weight growth between the higher and lower nitrogen levels, though the nitrogen of the latters was perfectly exhausted by 50 day's culture.

From these results, it can be concluded that the absorption of nitrate and the juvenile growth of *Chrysanthemum coronarium* var. *spatiosum* are, to some extent, independent of the nitrate concentration in water culture solution, but the maximal growth is limited by the amount of nitrogen absorbed. Similar trends were observed in phosphate and calcium experiments, whose results will be reported in another paper. Recently, Olsen^{12, 13, 14} found that ion absorption by rye and kitchen kale is independent of concentration above 0.003 m.eq. per liter.

b) **Chemical contents in plant tissues:** Nitrogen concentrations in leaves, stems and roots were investigated at successive stages of growth. The nitrogen levels in the tissues decreased with growing of the plants, and the decrease occurred in earlier stage in the plants at lower levels than in those at higher levels (see Fig. 3). This may suggest, in comparison with the growth curves in Fig. 1, that the plants can continue to grow for a few days at the cost of nitrogen reserved in the tissues, after the whole ions in the medium was absorbed by plants. Moreover, each organ had its critical nitrogen concentration below which its growth should be stopped. The critical concentrations were about 1.4 % in the leaves, 0.7 % in the stems, 1.2 % in the roots. The values may be very useful for the estimation of nutritional status of field-grown plants, as discussed by Lundegårdh⁷ and Joham⁶.

The results concerned with changes of total phosphorus are summarized in Table 1.

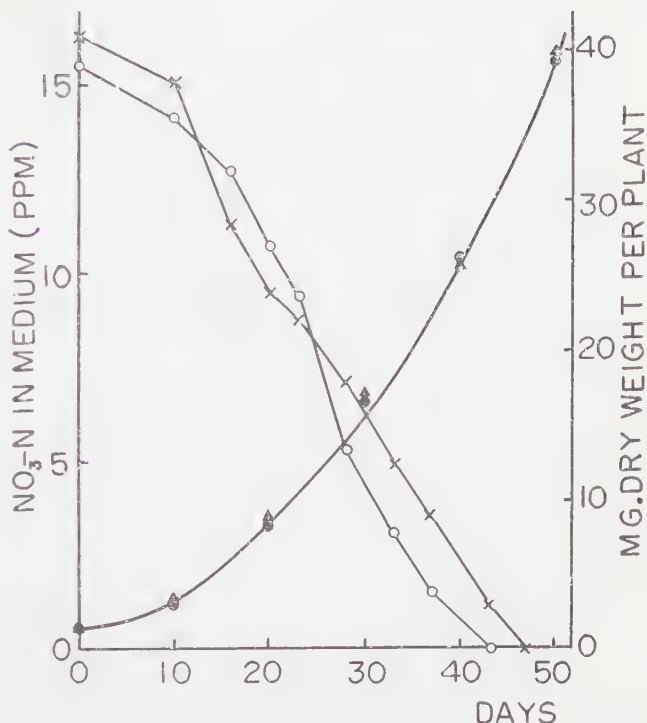


Fig. 2. Changes of nitrate nitrogen in medium and plant growth.

—▲—: control (150 p.p.m.) plant, —●—: experimental plant.
 —×—: medium containing 16.2 p.p.m. of nitrogen.
 —○—: medium containing 15.5 p.p.m. of nitrogen.

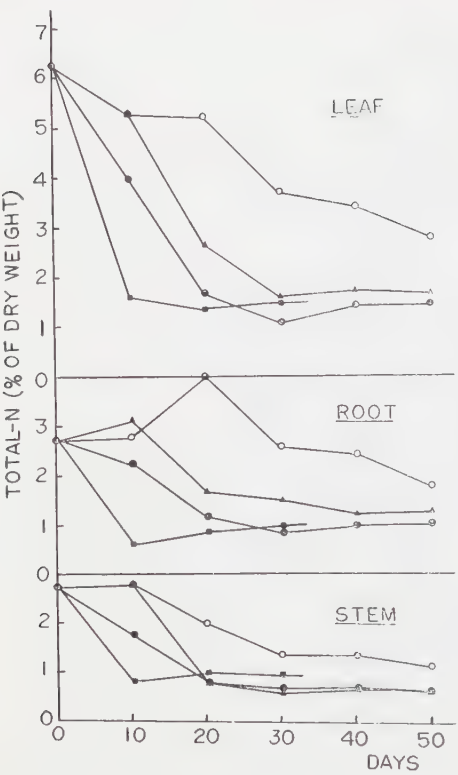


Fig. 3. Contents of total nitrogen at successive stages of growth.
○: N-100 plant, ▲: N-20 plant, ●: N-5 plant, ■: N-0 plant.

there remained low photosynthetic activity in optimal illumination. Such recession of photosynthetic activity has also been detected by Müller^{9), 11)} in *Sinapis alba*.

Respiration of leaves of the N-20 plants showed a low value in comparison with

Table 1. Phosphorus contents at successive stages of growth. (Per cent on dry weight basis)

	Nitrogen level	10 days	20 days	30 days	40 days	50 days
Leaves	N-5	0.70	0.54	0.19	0.33	0.45
	N-20	0.97	0.46	0.32	0.32	0.46
	N-100	0.74	0.70	0.13	0.15	0.17
Stems	N-5	—	—	0.11	0.26	0.33
	N-20	—	0.45	0.15	0.24	0.35
	N-100	—	0.38	0.08	0.13	0.12
Roots	N-5	—	0.58	0.11	0.21	0.51
	N-20	—	0.68	0.10	0.23	0.26
	N-100	—	0.61	0.08	0.20	0.16

Nitrogen deficiency resulted the accumulation of total phosphorus in each organ. Concerning ash, calcium and iron content, however, no marked differences were shown in connection with nitrogen deficiency, except for ash contents in stems and roots (Table 2).

Symptom of nitrogen deficiency was visual in chlorosis of leaves, especially of lower leaves, before whole plant growth retarded. This agrees with Müller's results¹⁰⁾. Chlorophyll content was 4.93 mg. per 10 g. fresh leaves of the plants at 20 p.p.m. nitrogen level (N-20), while of the at 100 p.p.m. levels (N-100) it was 6.20 mg., on the 30th day of culture.

c) **Photosynthesis, respiration and dry matter production.**— Photosynthetic activities of leaves of the N-20 and N-100 plants water-cultured for 30 days are shown in Fig. 4. It shows a marked recession in activity caused by nitrogen deficiency. At that time the growth of N-20 plants was already stopped, though

Table 2. Contents of ash, calcium and iron of N-100 and N-20 plants after 50 days. (Per cent on dry weight basis)

Organ	Nitrogen level	Ash	Calcium	Iron
Leaves	N-100	17.82	1.11	0.099
	N-20	17.29	1.86	0.080
Stems	N-100	19.55	1.86	0.025
	N-20	13.92	1.81	0.027
Roots	N-100	14.52	0.62	0.122
	N-20	12.40	0.50	0.134

that of the N-100 plants (see Table 3). Gregory and Richards⁵⁾ have also found the decreased respiration by 20 % in nitrogen deficient barley leaves, while Müller⁹⁾ described some increase of respiration in *Sinapis alba*. Nitrogen deficiency caused slight increase in respiration of the stems, and decrease in that of the roots as shown in Table 3.

Furthermore, calculation of dry matter production per day of these plants was done, according to Boysen Jensen's method. Gross production (P_g) of a plant was calculated as assimilated glucose from the data of light-photosynthetic curve at 25°, total leaf area and light intensity curve of a day of August at Tokyo. Total amount of respiration (R) of non-photosynthetic organs (stem and roots) per day was also calculated as consumed glucose at 25°. Net production (P_n) of a plant, i. e. the value of ($P_g - R$), was

Table 3. Respiratory activities of each organ of N-20 and N-100 plants. Temperature 25°.

	Leaves (mg. CO ₂ /50 cm. ² /hr.)	Stems (mg. CO ₂ /100 mg. D.W./hr.)	Roots (mg. CO ₂ /100 mg. D.W./hr.)
N-20	0.68	0.36	0.23
N-100	0.71	0.23	0.27

13.7 mg. in a N-100 plant on the 30th day, while that in a N-20 plant was rather -5.5 mg., so the relative daily net production of the former was 7.1%, and that of the latter -5.4% (see Table 4). These values correspond roughly to the gradients (7 %

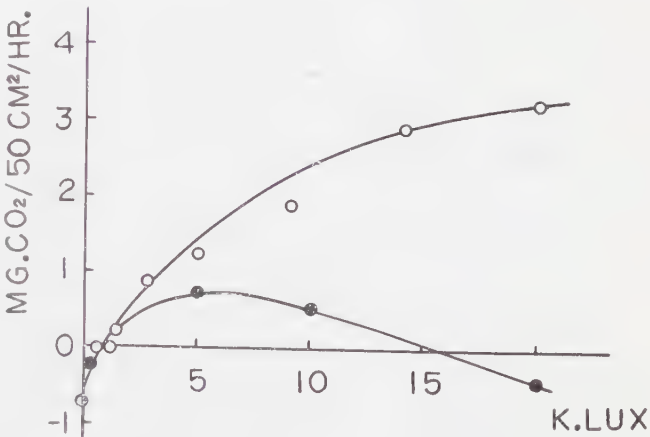


Fig. 4. Light-photosynthetic curves of N-100 and N-20 leaves after 30 days.

—○—: N-100, —●—: N-20.

Table 4. Daily production of a N-20 and a N-100 plant after 30 days (at 25°).

	Dry weight (mg.)				Leaf area (cm ² .)	P _g (mg.)	R (mg.)	P _n (mg.)	% P _n (mg.)
	Leaves	Stem	Roots	Total					
N-20	60	25	17	102	18.4	-3.2	2.3	-5.5	-5.4
N-100	124	44	25	193	37.3	16.8	3.1	13.7	7.1

and -3% respectively) at the 30th day point on the growth curves in Fig. 1. Müller⁹⁾ computed 27.6% net production in normal plant and 3.3% in nitrogen deficient one of *Sinapis alba* (at Copenhagen in July).

Summary

Influence of nitrate ions upon the growth of *Chrysanthemum coronarium* var. *spatiosum* was investigated by means of water culture, with special reference to dry matter production.

1) Absorption rate of nitrate ion and the juvenile growth of plants were almost independent of nitrate concentration of the medium. However, the maximal growth of plants was limited by the total amount of nitrogen available.

2) Nitrogen deficiency caused chlorosis, or chlorophyll content depression, in leaves, and accumulation of phosphorus in tissues, while it had no special effect on the content of ashes, calcium and iron.

3) Decrease of nitrogen concentration in tissues was persued at successive stages of growth. Critical nitrogen concentration in each organ, at which the growth should cease, was detected.

4) Marked recession of photosynthetic activity, and reduction of respiration in leaves and roots, but the increase of it in stems were observed being accompanied with nitrogen deficiency. Decrease of plant growth was also explained in the light of dry matter production.

The author wishes to express his deep gratitude to Dr. K. Hogetsu, Prof. of Tokyo Metropolitan University, for his kind encouragement and advice in the course of this work. Thanks are also due to Dr. M. Monsi, Prof. of University of Tokyo for many valuable suggestions.

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Physiological Studies on Growth and Morphogenesis of the Isolated Plant Cell Cultured *in vitro* V. Artificial Control of the Morphogenetic Polarity

by Tadashi SANDAN*

山段 忠*: 遊離植物細胞の生長成形に関する生理学的研究 V.
成形極性の人為的支配

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An isolated internodal cell of *Nitella* or a cell fragment obtained from the internodal cell by strangulation was able to develop a new shoot and rhizoids when it was cultured in agar gel with suitable culture solutions. Furthermore, the morphogenetic polarity was observed in the course of the development. Namely, a new shoot arose from the apical end of the cell or the cell fragment and new rhizoids appeared from the basal end in general. The term of the apical end represents the upper end of the cell when the cell took its position within the mother plant before it was cut off to be used in the experiment, and the term of the basal end indicates the opposite, lower end of the cell. But the morphogenetic polarity was reversed by changing light condition (Sandán, 1955). The present report deals with further experiments in regard to the artificial control of this morphogenetic polarity in the cell of *Nitella*.

Material and Method

An isolated internodal cell of *Nitella flexilis* which was about 4.0 cm. in length and 400 μ in width was applied as material. The material was set in the glass apparatus consisting of two chambers in which the cell is cultured under pH gradient or IAA concentration gradient. This apparatus which was designed by Prof. N. Kamiya has been used for studies of protoplasmic streaming in Myxomycetes and for studies of osmosis in a single cell of Characeae. In the present work, these two chambers in the apparatus (A and B in Fig. 1) were filled with 0.6 % agar gel which was soaked with culture solution. As culture solution, solutions of various pH and of IAA in various concentrations were used in the present experiment. In the case of pH, McIlvaine's citrate-phosphate buffer solutions (0.01 M citrate, 0.02 M phosphate) of various pH were used, and in the case of IAA, Sørensen's phosphate buffer (pH 6.6, 0.01 M) was applied as basic solution (control). The gradient of pH or IAA concentration was attained in the apparatus by using agar gel which was soaked

* Biological Institute, Kyoto Liberal Arts University, Fukakusa, Kyoto, Japan. 京都学芸大学生物学教室

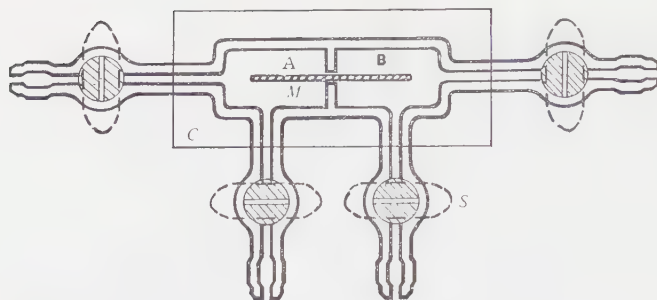


Fig. 1. The double chamber apparatus

A: A chamber (length: 3.0 cm., width: 1.5 cm., height: 1.2 cm.),
 B: B chamber, C: cover glass, M: material, S: stop cock

with the solution of different pH or different IAA concentration in two chambers of the apparatus. In these two chambers the material, which is divided into two portions by the central glass septum as shown in Fig. 1, was submerged in agar gel. The space between the material and the glass septum was filled with lanolin. Thus, the material was cultured in the apparatus under pH gradient or IAA concentration gradient which was given in the surrounding medium. These two chambers of the apparatus were covered with a glass slip, and four stop cocks of the apparatus were also closed usually. Each experiment was carried out in the two cases: first, the case in which the apical end of the cell was set in A chamber, and second, the case in which the basal end of the cell was set in A chamber. All experiments were tried at room temperature under diffuse light of about 80 lux.

Results

The materials which were cultured under pH gradient or IAA concentration gradient as mentioned above were able to grow and develop a new shoot and rhizoids. According to the results attained by the present experiment the polarity of morphogenesis, was different from the natural one. A new shoot and rhizoids arose from the end which was placed in more favourable medium whether this end was apical or basal.

1 Effect of pH gradient

Figure 2 shows the end from which a new shoot and rhizoids were formed in the cell that was cultured under pH gradient. In this figure, (S) represents shooting and (R) indicates rhizoid formation. For example, in Experiment 1 (Combination pH 4.0: pH 6.6) of this figure it is recognizable that a new shoot and rhizoids arose from the end of the cell portion which was placed in the medium buffered at pH 6.6.

In Control and Experiment 5, shooting generally took place at the apical end and rhizoid formation occurred at the basal end according to the natural polarity. In the other case, however, the natural polarity disappeared. Judging from the results, it seems that a new shoot and rhizoids are formed from the end which is

	A	B
Control	pH 6.6 (S or R)	pH 6.6 (R or S)
Experiment 1	4.0	6.6 (S) (R)
Experiment 2	5.0	6.6 (S) (R)
Experiment 3	7.6	6.0 (S) (R)
Experiment 4	5.0	7.6 (S) (R)
Experiment 5	7.6 (S or R)	6.6 (R or S)

Fig. 2. Shooting and rhizoid formation in the cells which were cultured under pH gradient

placed in the medium at or around pH 6.6.

The rate of the protoplasmic streaming in the cell was generally constant except for two or three days at the start of cultivation, when it decreases, and for several days prior to shooting, when it increases, in all cases. And there was no difference in the rate of streaming between the portion that was placed in A chamber and the portion in B chamber. The decrease in the rate of streaming is probably due to the mechanical shock accompanying the culture treatment. The time required by the material for shooting or forming rhizoids after it was brought into the culture medium, and the constant velocity of the protoplasmic streaming in each case are summarized in Table 1.

Table 1. Time required by the cells, which were cultured under pH gradient, for shooting or rhizoid formation, and the constant velocity of the protoplasmic rotation

	Time for shooting (days)	Time for rhizoid formation (days)	Constant velocity of protoplasmic rotation (μ /sec)
Control	16	17	58
Experiment 1	20	20	48
Experiment 2	19	20	51
Experiment 3	21	22	44
Experiment 4	22	23	41
Experiment 5	19	18	53

2 Effect of IAA concentration gradient

Figure 3 shows the end at which the new development occurred in the cell that was cultured under IAA concentration gradient. In Control, Experiment 1 and Experiment 5, a new shoot was formed from the apical end of the cell and rhizoids arose from the basal end according to the natural polarity. However, the natural polarity was lost in other cases. Considering the results, it seems that a new shoot and rhizoids are ready to be formed from the end which is placed in the medium containing about 0.1 mg./l. IAA.

Similarly to the case in which pH gradient was applied, the rate of protoplasmic streaming in the cells which were cultured under IAA concentration gradient was generally constant except for two or three days at the start of cultivation and for several days prior to shooting. However, in Experiments 1, 2 and 3 the rate of flow

	A	B
Control	0 mg./l. (S or R)	0 mg./l. (R or S)
Experiment 1	0 (S or R)	0.05 (R or S)
Experiment 2	0	0.1 (S) (R)
Experiment 3	0	5.0 (S) (R)
Experiment 4	0.1 (S) (R)	5.0
Experiment 5	0.1 (S or R)	0.5 (R or S)

Fig. 3. Shooting and rhizoid formation in the cells which were cultured under IAA concentration gradient

Table 2. Time required by the cells, which were cultured under IAA concentration gradient, for shooting or rhizoid formation, and the constant velocity of the protoplasmic rotation

	Time for shooting (days)	Time for rhizoid formation (days)	Constant velocity of protoplasmic rotation (μ/sec.)
Control	16	17	58
Experiment 1	17	17	58
Experiment 2	19	18	56
Experiment 3	20	19	50
Experiment 4	19	21	52
Experiment 5	17	18	56

in the portion which was placed in B chamber (containing IAA) was slightly larger than that in the portion in A chamber (containing no IAA) for three or four days after recovery to the normal rate from the diminished rate, which is probably due to the mechanical shock accompanying the culture treatment. But the velocity of the portion in B chamber soon became slow and reached the same value as that of the portion in A chamber. The time required by the materials for shooting or forming rhizoids and the constant velocity of the protoplasmic rotation are shown in Table 2.

Discussion

A new rhizoid was formed from the portion in the medium of strong acidity when the fertilized egg of *Fucus* was placed in the medium in which pH gradient was given: pH 5.8 at one side of the egg and pH 8.3 at the opposite side (Whitaker, 1937). In the present work using *Nitella* cell, a shoot and rhizoids arose from the end which was placed in the medium at pH 6.6 or around 6.6. Sandan and Ogura (1957) reported that the most suitable pH for the morphogenesis of the cell of *Nitella* is around 6.6. Olson and duBuy (1937) observed that when fertilized egg of *Fucus* was placed near a capillary which was filled with sea water containing β -IAA, a new rhizoid arose from the portion near the capillary. Overbeek (1940) mentioned that rhizoid formation in various algae ordinarily arises at the portion containing a large amount of IAA. Bünning (1952) said that the factor controlling polarity in plant may be unequal distribution of IAA.

According to the results obtained from the present work, a shoot and rhizoids in the *Nitella* cell, which was cultured under IAA concentration gradient, were formed from the end that was placed in the medium containing about 0.1 mg./l. IAA. The appropriate concentration of IAA for the morphogenesis of the cell is 0.1–0.3 mg./l. (Sandan and Ogura, 1957). Thus, a new shoot and rhizoids in the isolated cell of *Nitella*, which was cultured under pH gradient or IAA concentration gradient, arose from the end that was placed in adequate medium, whether this end was apical or basal.

The morphogenesis and the protoplasmic streaming in *Nitella* cell were accelerated by application of IAA in low concentrations (Sandan and Ogura, 1957). In the present work, however, the velocity of protoplasmic streaming in the cells which were cultured under IAA concentration gradient was not larger than that in the control cell. Further, in the case of control, a shoot and rhizoids were formed earlier than in all the other cases. It is likely that the cell is liable to be hurt when its two ends are brought into contact with solutions of IAA of different concentrations even though each of the concentrations is favourable for morphogenesis. This is also true for pH.

Artificial control of morphogenetic polarity in *Nitella* cell was also attained by the experiment of Sandan (1955) who found the following phenomena: When the isolated internodal cell of *Nitella* was cultured in the vertical, normal position, a new

shoot generally arose from the apical end and rhizoids appeared from the basal end, but when the cell was cultured in the vertical, inverted position and the lower half of the cell was kept dark, a new shoot was formed from the upper end, which is actually the basal end, and rhizoids arose from the lower end, which is actually the apical end.

It has been well known that rhizoid formation of spore cell in such lower plants as *Equisetum*, *Cystoseira*, *Anthoceros*, *Laurencia*, *Marchantia*, *Fucus* and *Bryopsis* occurs at the dark portion of spore cell (Stahl, 1885; Peirce and Randolph, 1906; Hund, 1931; Steinecke, 1925; Knapp, 1931; Bloch, 1934; White, 1934).

In the light of the present experiment and of the previous work with special reference to the reversal of morphogenetic polarity by changing light condition (Sandan 1955), we are now in a position to say that the morphogenetic polarity in *Nitella* cell is certainly weak and is easily controlled by artificial treatment.

Summary

When the isolated internodal cell of *Nitella flexilis* was cultured under pH gradient or IAA concentration gradient, which was given in culture medium, a new shoot and rhizoids were formed from the end which was placed in more adequate medium, whether this end was apical or basal. Sandan (1955) found that the morphogenetic polarity of the cell can be reversed by changing light condition. In the light of these experiments we are now in a position to say that the morphogenetic polarity in the cell of *Nitella* is certainly weak and is easily controlled by artificial treatment.

The author wishes to express his most cordial thanks to Prof. N. Kamiya of Osaka University for his kind direction and helpful criticism throughout this work and also to Prof. T. Nakamura for his valuable advice.

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On the Anthocyanin in the Hair of Spike of Japanese Pearl Millet*

by Mannen SHIBATA** and (Miss) Emi SAKAI**

柴田萬年**・堺 恵美**: チカラシバ花穂の毛のアントシアニンについて

Received February 21, 1958

Japanese pearl millet, *Pennisetum japonica* Tri., which belongs to Gramineae, is a perennial herb growing vigorously in the field or on the roadside. In early autumn it bears an about 17 cm. long cylindrical spike at the top of the culm with reddish purple hair which becomes black purple in late autumn and discolours in winter. A preliminary examination indicated that the colouring matter in the hair is an anthocyanin.

The present study is to further the investigation and determine precise nature of the anthocyanin.

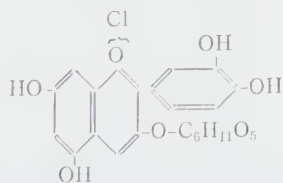
In 1955 one of us (M.S.) succeeded in obtaining in crystalline state the pigment in the hair of the spike of this grass collected on the campus of our university, but the yield was too small for the determination of the precise nature of this anthocyanin.

The process of isolation of the pigment from the spikes collected on the campus in mid-October, 1956, is described in detail in the experimental part.

The yield of the crystalline pigment was 728.1 mg., about 0.002 per cent of the fresh weight of the collected spike, the comparative scantiness of the yield being attributable to the fact that the pigment is contained only in the hair of the spike.

The results of the investigation showed that the crystalline pigment was a glucoside, which consisted of cyanidin and glucose. The pigment was, therefore, supposed to be either chrysanthemin or cyanin.

As a result of comparative studies of the pigment of this grass with chrysanthemin, which had been isolated from the flower of *Lycoris radiata* Herb. by K. Hayashi (1937), it was concluded that the pigment of the spike of *Pennisetum japonica* Trin. is nothing other than chrysanthemin (3-β-glucosidylcyanidin chloride).



Chrysanthemin

* This report was presented at the 75th Anniversary Meeting of the Botanical Society of Japan, held on Oct. 12-15, 1957, in Tokyo.

** Biological Institute, Toyama University, Toyama, Japan. 富山大学文理学部生物学教室.

Experimental

Isolation and purification of anthocyanidin glucoside.

In a percolator were placed 32.445 kg. of the fresh spikes of *Pennisetum* and were immersed in 21 l. of 1% methanolic hydrochloric acid. After being allowed to stand overnight, the pigment solution was decanted. Then the residual spikes were soaked twice in 8 l. of methanol, taken out, enclosed in a hemp bag and pressed. The extract obtained was filtered.

About 36 l. of brown-red extract thus obtained was mixed, under continuous stirring, with saturated basic lead acetate solution, whereupon first white precipitate of lead chloride, then green precipitate of lead compound of anthocyanin was formed. The precipitate was filtered by suction, washed thoroughly with water, then with absolute ethanol and dried in a calcium chloride-containing desiccator (1025 g.). The lead compound of the pigment was pulverized in a mortar and converted to chloride with 5% methanolic hydrochloric acid. Then the white precipitate of lead chloride was filtered off. The bluish red filtrate obtained was carefully concentrated to 2/3 volume under a reduced pressure at 35° and filtered. The concentrated filtrate of the pigment was precipitated by the addition of 5 volumes of ether and kept in a refrigerator overnight. The hygroscopic precipitate, which adhered to the wall and the bottom of the flask, was dissolved in a small quantity of ethanol and then filtered. The filtrate began to precipitate on addition of 3 volumes of ether and it was kept in a refrigerator to complete the precipitation. The process was repeated three times till the amorphous precipitate of the pigment was no longer hygroscopic. The purified precipitate was dissolved in a small quantity of absolute ethanol and then the solution was filtered. To the filtrate, which contained the purified anthocyanin chloride, 1/2 volume of 5% ethanolic hydrochloric acid was added. After being allowed to stand for 5 days at room temperature, the solution began to form the crystals of the pigment. Then it was stored in a refrigerator for completion of the crystallization. After 11 days of incubation about 0.8031 g. of raw crystals of anthocyanin were obtained. Then a small amount of cold 5% ethanolic hydrochloric acid was added to the mother liquor, which was allowed to stand several days at room temperature and a little more crystals of about 0.28 g. of red pigment were obtained. The total weight of the raw crystals obtained was, therefore, about 1.831 g.

Raw crystals of the pigment dissolved in a small amount of warm water, on addition of an equal volume of 7% hydrochloric acid, formed lens-shaped purplish crystals after being allowed to stand for 3 days, but, on addition of an equal volume of 5% ethanolic hydrochloric acid, formed red-brown needles after being allowed to stand for a day. After two more process of recrystallization, 0.7281 g. of pure preparation was obtained. The yield was very low (about 0.002% of the fresh weight).

Anthocyanin chloride (Chrysanthemin chloride)

The crystals of *Pennisetum*-anthocyanin obtained, as shown in Fig. 1, were purple,

of small lens shape and had a fine golden luster. The air-dried preparation decomposed at 221° (uncorr.), had $1\frac{1}{2}$ molecule of water of crystallization and lost all of it at 105° *in vacuo* (1 mm. Hg).

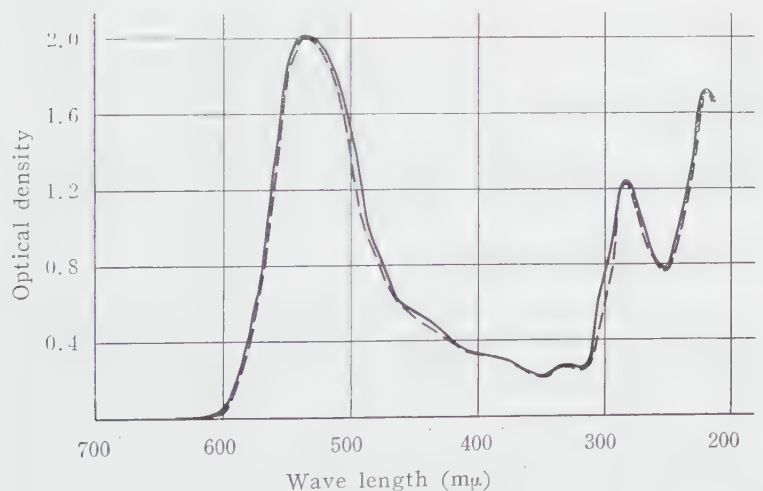
They were easily soluble in water and methanol giving a blood-red solution, slightly soluble in ethanol, 0.5% and 20% hydrochloric acid, insoluble in 1, 2, 3, and 7% hydrochloric acid and easily soluble in 7% sulphuric acid. The pigment solution, dissolved in a small amount of warm water and diluted with a large amount of ethanol, showed the following colour reactions: it turned blue on addition of sodium hydroxide solution, turned red purple with sodium carbonate solution and pure blue with ferric chloride solution.

The distribution number of this pigment was estimated as 10.5 and 12.0, somewhat different from that of the chrysanthemin from *Lycoris* (18.6, 18.1) (K. Hayashi, 1937), from *Calycanthes* (10-12) (K. Hayashi, 1952) or obtained from red autumn leaves of *Acer* species (17.8, 19.0) (S. Hattori and K. Hayashi, 1937).

The *Pennisetum*-anthocyanin was compared with the authentic specimen of chrysanthemin from *Lycoris radiata* Herb.

No depression of the melting point was observed in the mixture of chrysanthemin (222°) and *Pennisetum*-anthocyanin (221°).

Paper chromatography by the ascending one-dimensional procedure was achieved



— "Pennisetum"-anthocyanin, 5/10,000 mol in 60% EtOH (HCl-conc. 0.1%).
 - - - Chrysanthemin (from *Lycoris radiata* Herb.), 5/10,000 M. in 60% EtOH (HCl-conc. 0.1%).

Fig. 2.

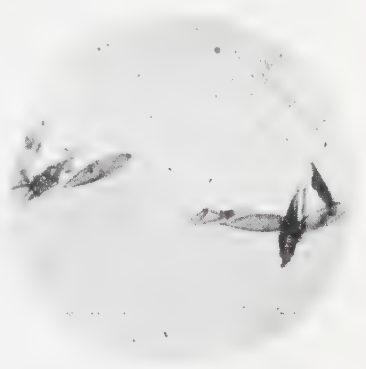


Fig. 1.

at room temperature with acetic acid/conc. hydrochloric acid/water mixture (3:1:8), *n*-butanol/acetic acid/water mixture (4:1:5), and *n*-butanol/conc. hydrochloric acid/water mixture (7:2:5) using Tōyō No. 52 filter paper. The results of paper chromatography, with acetic acid/conc. hydrochloric acid/water mixture (3:1:8) as a de-

veloping solvent showed the following R_f -values:

Chrysanthemin from *Lycoris* 0.37(1), *Pennisetum*-anthocyanin 0.37(2), the mixture of the pigments 0.37(2).

Comparison of the absorption spectra of chrysanthemin from *Lycoris* and *Pennisetum*-anthocyanin showed that both of the curves exhibited characteristic peaks at 530, 283 and 210 $m\mu$ coinciding with each other (Fig. 2).

Anal. Calcd. for $C_{21}H_{21}O_{11}Cl$: C, 52.03; H, 4.37. Found: C, 52.33, 52.42; H, 4.55, 4.36.

Water of crystallization. Calcd. for $C_{21}H_{21}O_{11}Cl \cdot \frac{1}{2} H_2O$: $\frac{1}{2} H_2O$, 5.28. Found: H_2O , 5.32.

The experimental results described above leave no doubt that the *Pennisetum*-anthocyanin was essentially the same as chrysanthemin obtained from the flower of *Lycoris radiata* Herb.

Hydrolysis of *Pennisetum*-anthocyanin chloride

In 30 ml. of warm water were dissolved 146.2 mg. of air-dried crystals of *Pennisetum*-anthocyanin, to which was added an equal volume of conc. hydrochloric acid (d.1.18). The solution was boiled for 3 minutes and allowed to stand for a day in a refrigerator. Dark chocolate-brown needles (aglycone) crystallized out from the solution, the mother liquor becoming faintly rosy in colour. The crystals of aglycone were filtered by suction, washed with 10% hydrochloric acid and dried over sodium hydroxide in a desiccator. The aglycone obtained was about 96.5 mg. in weight, the yield being 66.0 per cent of the anthocyanin used.

The acidic mother liquor was shaken thoroughly with *iso*-amyl alcohol to remove a trace of dissolved aglycone. The mother liquor was then shaken with ether to remove *iso*-amyl alcohol dissolved.

The nature of the sugar contained in the solution was tested by colour reactions, paper chromatography and osazone formation.

As a developing solution of chromatography, mostly the mixture of *n*-buthanol/acetic acid/water (4:1:2) and sometimes that of phenol/water (6:1) was used and the developed filter paper was sprayed with 0.1% potassium permanganate solution or ammoniacal silver nitrate solution. There appeared only a spot of glucose on the paper chromatogram.

The osazone of the sugar was obtained by the usual procedure in yellow acetone-insoluble needles and had a melting point of 205°. The osazone was mixed with authentic glucosazone (m.p. 206°) and no depression of the melting point was observed.

The data for the determination of the sugar described above indicate that the glucose is the sole sugar combined with the anthocyanin pigment in the hair of the spike of *Pennisetum*.

Aglycone (Cyanidin chloride)

The aglycone obtained by hydrolytic decomposition of *Pennisetum*-anthocyanin

was dissolved in a small amount of ethanol and filtered, and then nearly an equal volume of 2% ethanolic hydrochloric acid was added to it, whereupon long red brown needles crystallized out from the solution (Fig. 3). The crystals presented the same appearance as described in detail in the report of R. Willstätter and E.K. Bolton (1916). They lost a molecule of water of crystallization under a reduced pressure of 1 mm. Hg at 105° and showed the following characteristics: they were easily soluble in ethanol and methanol; almost insoluble in water and in cold dilute and concentrated hydrochloric acid; fairly soluble in warm 7% sulphuric acid and when cooled, crystallized out as the sulphate of cyanidin. The aglycone dissolved in dilute hydrochloric acid was extracted completely with *iso*-amyl alcohol. The purplish alcohol solution of cyanidin became pale yellowish brown (formation of carbinol base) on addition of 2 volumes of hot water, and on addition of acid turned slowly again to initial purple.



Fig. 3.

On addition of ferric chloride solution, the ethanol solution of aglycone turned blue, and then dirty green; on addition of 20% lead acetate it formed a blue precipitate; turned purple with 50% sodium acetate and blue with 50% sodium carbonate. The aglycone showed the absence of a methoxyl group after Zeisel-Pregl method.

Anal. Calcd. for $C_{15}H_{11}O_6Cl$: C, 55.81; H, 3.41. Found: C, 55.60; H, 3.46.

Water of crystallization. Calcd. for $C_{15}H_{11}O_6Cl \cdot H_2O$: H_2O , 5.29. Found: H_2O , 5.45.

From the above mentioned experimental results, one might conclude that the aglycone of *Pennisetum*-anthocyanin is nothing other than cyanidin.

We are indebted to Prof. K. Hayashi (Tokyo University of Education) for the valuable advice and the kind supply of the authentic specimen of chrysanthemin.

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抄 録

George Tischler u. Heinz D. Wulff 1953-1958.

Angewandte Pflanzenkaryologie, 127 図, 全6分冊 (1180 頁予定)

本書は G. Tischler: Allgemeine Pflanzenkaryologie 第2版 (1951: 1040 頁) の別巻である。“一般植物核学”は初版が 1921~1922 に刊行され、第2版の改訂は 1944 年に着手せられ、1951 年に刊行された。応用植物核学の刊行のいきさつは“一般植物核学”第2版の序文にかかれている。

第一次大戦のだいぶ前のある日、ドイツ植物学会である先輩からお前はまだ細胞核の問題をやっているのか、“Mode von vorgestern,” 時代おくれじゃないかと言われて面くらったが、自分も若かったので“Oder von übermorgen”と反撥の気持を含めて答えたことを憶えている。私は既に 65 才 (1944) となったが、私の予言は正しかったことを嬉しく思っている。実を言うと、その当時、植物核学がこれ程の大発展をとげようとは思わなかった。第2版に引用した文献は8767篇であるが、核学を基礎とした応用研究の分野は極めて広く、この“一般植物核学”を際限もなく膨大なものにすることは不可能であるので、初版の第9章に収めた部分、すなわち、Die Chromosomen und ihre Bedeutung für Stammes- und Erblichkeitsforschung に相当する分野は割愛し、別に関連諸問題をあわせて別巻 Angewandte Pflanzenkaryologie として刊行する”と述べている。

この別巻は1953年に第1分冊 (208 頁) が刊行され、1956 年、第5分冊を以て完結予定であった。1954年7月パリに開かれた万国植物学会に元気な姿を見せていた Tischler 教授は、同年12月末日、突然逝去した。このため、予定はだいぶ遅れた。しかし幸に高弟 Heinz Diedrich Wulff 教授が第3分冊以降を引ついで、Tischler 教授の遺業をまさに完結せんとしている。1954年以後、毎年1分冊、208 頁づつを刊行、1957年分第5分冊 (849-1072頁) まで刊行済みである。第6分冊 (文献目録と索引) は1958

年初頭発行予定。

目次: 1. 核学と遺伝学との関係 2. 染色体の化学と構造、特に遺伝子の本性とその座 3. 形質の発現 4. ゲノム突然変異 5. 染色体突然変異 6. 永久雑種 7. 真性雑種とその不稔現象 8. プルドーに関する問題 9. 種並に属の成生 10. 系統学に対する染色体研究の意義 11. 核学と生態学並に植物地理学との関係 12. 追補 13. 文献 (本文 775 頁, 以下文献目録と索引)

一般植物核学は核分裂と核融合に関する分野だけでいわば静的核学であるのに対し、本書は細胞核の代謝、遺伝子の生理生化学的性状、遺伝子の座としての染色体の電子顕微鏡的微細構造、遺伝子群の担荷体である染色体の構造的、数的質的变化による細胞の、あるいは生物の変化、細胞質による遺伝子発現の変更、異質細胞質による染色体数の変化などを緯とし、動植微生物全般にわたる豊富な事例を経として記述された動的な核学である。これは核学を基礎とした遺伝学の集大成である。ドイツ的という言葉に全くふさわしい著書であり、そして文字通り Tischler 教授が75年の生涯をかけた畢生の大著であり、斯学に遺産として残した偉大な記念塔と称すべきものである。

全く類書がないと断言できるこの大著に数多くの日本人の業績が寄与していることはわれわれの誇りとしていたいところであると同時に、われわれは Tischler 教授が本書を通じて後進に遺した多くの示唆をかみしめて指針としたいものと思う。

Wulff 教授の努力によって1956年1月までの追補が添えられたことは本書の価値を更に高めたものと言えよう。引用文献は約 14000 篇にのぼっている。(Gebrüder Borntraeger-Berlin-Nikolassee, 予約価第1-5分冊 DM 201, 第6分冊 DM 30)

(田中信徳)

雑 録

植物の蒸散作用の検出について*

Detection of Transpiration of Moisture by Plants*

by

Arcot Viswanathan** and Syed Azmathullah***

植物から蒸散した水蒸気が非常に興味ある化学反応を起こす実験をこれから述べよう

二枚の完全に円形のセロファン紙、あるいは光沢のある薄いカード紙（直径おのおの 25 cm.）を、ちょうどロ紙で漏斗を作るようなぐあいに漏斗状におりまげ、形がくずれないようにピンでとめておく。精製した乾燥アルミニウム粉末(0.5g.)と、昇華した乾燥ヨウ素結晶(0.75 g.)とを乾いた乳鉢内で急入りに混合し、スレート板、平らな陶器板、あるいはセメントアスベスト板(幅 5cm.)に等量に分けて盛りあげ、その上におのの漏斗をかぶせる。実験材料として、根の上部で切りとったばかりの新鮮な植物、例えばテンシクアオイやハウセンカを洗わずに用いる。5~6 枚の葉を一方の漏斗の中に入れて、葉を漏斗の先端の小さな穴から出し、水の入っている、適当な角度で固定した試験管の中にしこむ。漏斗は下方にそっとおきしておく。

数分後、葉から蒸散した水蒸気が漏斗の内側に溜まると、下にある薬品は紫煙を発生し、数秒後には白色の炎を出して燃えはじめる。この燃焼が起こる前に、反応がよく見えるように漏斗をとり去る。対照としておいてあるもう一方の薬品の方には何の反応も起らないが、それに水を一滴加えると燃えるから、水が化学反応を起こさせることがわかる。もし必要なら、同じような第三の薬品の山を作り、これには何も処理しないで置いて、それが水分を吸収しないときには何の変化も起こさないのを示すのもよいだろう。

アルミニウムとヨウ素とが反応してヨウ化アルミニウムを生成する時、水蒸気が触媒として働らく(1)。それゆえ公開実験を成功させるためには、

乳鉢も含めて、用いる材料はすべて使用する前に完全に乾燥させ、薬品の混合も実験直前に行なわなければならない(2)。雨天とか非常に湿度の高い日にこの実験を行なうのは不適當である。昇華したヨウ素数グラムを 3~4 日間、ゴム栓で口を閉じた試験管に入れて生石灰(酸化カルシウム)の上におき、乾燥させるのがよい。その時石綿せんいかガラス綿をつめて、石灰とヨウ素とを入れた試験管を離しておく。アルミニウム粉末は乾燥器のなかか、乾燥用円錐(drying cone)上で 110°で 1 時間乾燥させ、ヨウ素と同様に保存しておくのがもっともよい。このように乾燥して薬品を保存しておくと、実験にすぐ使用できる。セロファンのような透明な漏斗を用いると、実験のさい薬品の変化を最初から観察できる。ガラス製漏斗(直径約 12 cm.)もまた便利である。漏斗の柄は植物を入れることのできるように切りとっておかなければならない。

上に述べた範囲の量の薬品を使えば、実験はごく安全である。この公開実験は、脱水した塩化コバルト紙を用いる従来の方法よりも敏速に行なうことができ、また壮観であるばかりか、もっとも明瞭に、そしてもっとも確実に蒸散作用を示してくれる。

終りに当たり、著者(A. V.)は Dr. L. M. Yeddamnapalli; S. J. 教授が、寛大な便宜を与えられたことを感謝する。(戸塚 績訳)

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- 2) Ayed Azmathullah and Arcot Viswanathan, (1955) J. chem. Education 32: 447 (1955).

* この文章は原著者の依頼により紹介した。

** Loyola College, Madras-31, India.

*** The New College, Madras-14, India.

本 会 記 事

本会の図書幹事は金井弘夫氏に留任をおねがいしました。

支 部 通 信

北 道 海 支 部

4 月例会（4 月 26 日，於北大・農）館脇 操：フランスの印象 山田幸男：フランスの旅 なお支部長には館脇 操氏が再選されました。

第 11 回支部大会および総会は 6 月 14 日（土曜日）午前 9 時より，北大理学部において開催されます。なお特別講演として時田 郁氏の「欧米をめぐりて」と倉林 正氏の「集団遺伝」が予定されております。

関 東 支 部

32 年度支部大会（4 月 6 日，於東大・理）志平 依久子：ヒトエグサの成熟周期性の研究 I 石川 元助：トリカブトとアイヌの矢毒。佐藤正一：紡錘体の電子顕微鏡的研究。三井義蔵：綿毛細胞膜の原せんい系の研究。鈴木貞雄：東北および北関東におけるササ属の分布と生態について。高橋基生：根系呼吸曲線に表われた冷害の特質ならびにその植物分布決定に対する意義。百瀬静男：無配生殖をするシダについて 沢村正五：細胞原形質の形態変化と媒液の滲透価との関係。湯浅 明：コウボの核および核分裂。

中 部 支 部

第 6 回大会（4 月 10 日，於名市大・教養）川松重信：アカウキクサの根毛内の顆粒について。

太田敬久：2, 3 の植物の花粉母細胞でみられる核外染色粒について。市村国彦：アズキ幼胚の生長と電位分布。平松和幸，森 隆也：花粉の人工培地に用いる糖について 高木典雄：本宮御嶽山の蘚苔類植生 倉内 一：純イチヨウの個性性 脇田晴美：輸入穀物中に混入していたヒルガオ科植物種子。神谷 平：愛知県産フシナシミドロ属。須賀英文：愛知県産輪藻類についての 2, 3 の知見。谷口森俊：志摩英虞湾の海藻植生。大原準之助：三重県九鬼の植物について。高尾昭夫：ソラマメの胚発生組織化学的研究。熊沢正夫：前葉配列に関する続報。沢井輝男：不詳。岩塚 寿：Thiobacillus thiooxidans の sulfur oxidation について。鈴木 昇：ヒドラジンと窒素固定。

北 部 支 部

第 7 回総会および第 29 回例会（4 月 27 日・於県立福野高校）有馬忠雄：大麦幼植物における P³² 吸収におよぼす P, Fe 欠乏の影響。鈴木米三：ハシリドコロのポリフェノラーゼの研究 V，チオール化合物の阻害について。香室昭円：ヨシ沼沢地についての植物生態学的研究 III，優占群落と 2, 3 の土壌要因との関係について。

九 州 支 部

第 49 回例会（4 月 12 日，於九大・教養）中村和郎：アカパンカビにおける系統および温度による後還元分離開度の変動。宮本正一：昆虫と植物との関係，とくに虫糞について。

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71 卷 835 号 61 頁 Fig. 2

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Experimental

負となった細胞外

還元銀の白色

Experiential

負となった。細胞外

還元銀の黒色

Distribution of Electric Potential on the Seedling of *Phaseolus angularis*, a Hypogeal Plant.*

by Kunihiko ICHIMURA** and Hisashi OKAMOTO**

市村国彦・岡本尚**: アズキ幼胚上の電位分布について

Received January 22, 1958

During the past few years some investigations were carried out on distribution of electric potential on embryonal plants^{1,2)}. At our laboratory, distribution of electric potential in relation to growth process was investigated, using an epigeal plant, *Vigna sesquipedalis*. The results obtained by one of us²⁾ showed that a distinct valley of potential curve along the growing axis appears on the elongating part of the hypocotyl after 60 hours in culture, the relative position of the valley moving upward with growth of the plant and finally, in later stages of germination, shifting to the growing part of the epicotyl. It was pointed out that value of $\frac{\partial^2 E}{\partial x^2}$, the second spatial derivative of the electric potential (E) with respect to the elongation axis (x), is positive in the region where elongation occurs. Hatakeyama and Kawano³⁾ observed an appearance of a similar potential valley in the upper region of a shoot of sweet potato, where active growth is supposed to occur, though they described little about the growth process of the shoot.

We report in this paper results of investigations on distribution of electric potential on the hypogeal embryo of *Phaseolus angularis* and those on changes of the potential distribution during its growth process. Our aim was to determine whether the same relation between distribution of electric potential and growth of axial organ as found in *Vigna sesquipedalis*, exists in *Phaseolus angularis*, a typical hypogeal plant.

Material and Method

Seedlings of *Phaseolus angularis* were used for experimental material. After seeds were soaked in tap water for 8 hours at 30°, they were sown in washed sand and then incubated in the dark at 30° for 6 days.

There is a marked contrast in growth behavior between seedlings of *Phaseolus* and those of *Vigna*. The epicotyl of *Phaseolus* begins to grow after a short lag period of one and one-half days; during the next four and one-half days it continues

* Supported by the Grant in Aid of Scientific Research of the Ministry of Education.

** Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan. 名古屋大学理学部生物学教室

to grow rapidly, until, within six days after seed sowing, it has reached 210 mm. in length, whereas the hypocotyl grows rapidly up to 5 mm. only in the first day and one-half (a time during which the epicotyl has not yet started its growth), and from then the hypocotyl remains unchanged. As to *Vigna* seedling, the hypocotyl elongates rapidly in the first four days until it reaches a length of about 140 mm. when growth ceases and then the epicotyl begins to grow. Therefore, the essential difference between the epigeal and hypogeal growth is ascribed to the relative growing capacities of hypocotyl in both plants.

An electron tube potentiometer was employed for measuring the potential difference. For measuring electrode, a non-polarizable electrode of Zn/ZnSO_4 /Shive solution was employed.

The material was placed on keel-blocks composed of a number of agar pieces in a set. Details of this arrangement were described in the previous paper²⁾.

Except for the 2-day-old embryo, one electrode was always fixed at a point on the epicotyl about 10 mm. above the cotyledonary node and another electrode was put successively one after another along the elongation axis on each of the agar pieces of the keel-blocks. To pick up potential differences in the hook region, the arrangement of agar pieces was suitably modified from the regular serial arrangement of keel-blocks.

It was difficult to place the 2-day-old embryo on keel-blocks of agar pieces. For that reason agar pieces were arranged so as to be in contact with certain regions of the epicotyl.

Electric potential of the cotyledon was also measured. Seed coat was peeled off and a small cubic piece of agar was attached to the naked cotyledon. The measuring electrode was placed in contact with this agar piece. In the experiment, results of which are illustrated in Fig. 5, the reference electrode was attached to the cotyledonary node.*

These experiments were carried out at room temperature, from 25° to 30° under diffused light.

Results and Discussion

Longitudinal distributions of electric potential on the embryo of *Phaseolus angularis* at various culture stages are illustrated in Figs. 1-A to E.

Here a valley of potential curve appears clearly in the part of the epicotyl elongating most rapidly, in the same way as was formerly reported for the hypocotyl of *Vigna*²⁾. The relative position of the potential valley changes upward accompanying the growth of epicotyl. Elongation process of the epicotyl is very similar to that of the hypocotyl of an epigeal *Vigna* embryo (Fig. 2 and c.f.⁹⁾). A noticeable elongation of epicotyl occurs in the most apical region of 21-24 mm. in length (Zone A in Fig. 3), which is the hooked region.

* The potential difference between the cotyledonary node and the point 10 mm. above it is usually only 1-2 mV; in no case can it exceed 3 mV.

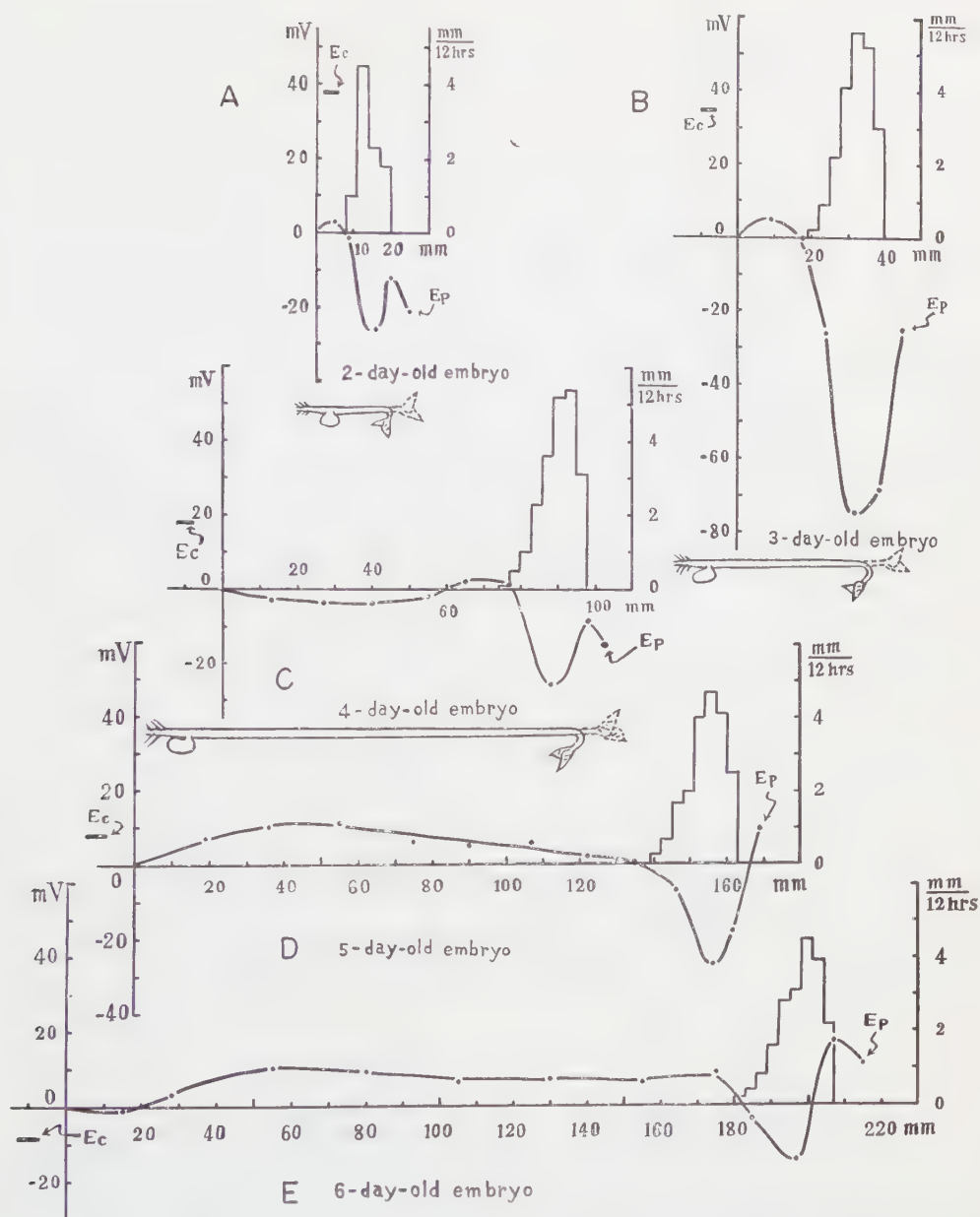


Fig. 1 A-E. Distribution of electric potential and elongation velocity of epicotyl in various culture ages.

Ordinates (left): Potential difference in mV.

(right): Elongation velocity of epicotyl in mm./12 hrs.

Abscissae: Distance in mm. from point of reference electrode, 10 mm. above cotyledon. (With 2-day-old embryo, the reference electrode is placed in the boundary region between radicle and hypocotyl.)

E_c : Potential level of cotyledon.

E_p : Potential level of plumule,

It was pointed out previously²⁾ that the distribution of elongation velocity does not coincide with the distribution of electric potential itself, but that it does with the distribution of the second spatial derivative, $\frac{\partial^2 E}{\partial x^2}$ of the electric potential (E) with respect to elongation axis (x), and that $\frac{\partial^2 E}{\partial x^2}$ always has a positive value in a region where elongation occurs. Theoretical basis for these phenomena was also discussed.

We can recognize that this hypothesis is applicable to the hypogeal embryo as well, viz., that the elongating region corresponds to the valley of potential curve where values of the second derivative are positive so far as the epicotyl of the 2- to 6-day-old embryo is concerned (Fig. 3, Zone B).

The fact that the head region of the epicotyl where elongation does not take place has a slightly higher potential level than the hook, can be ascribed to low water content or less consumption of substances, including various kinds of ions. This condition signifies that there is a condensed state of ions, which causes a valley in the potential curve in a region somewhat apart from the head region.

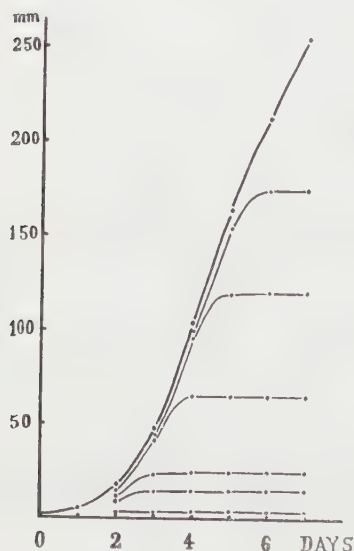


Fig. 2. Growth curves of epicotyl for the different parts of the axis.

Each curve represents the position of a growth mark made at certain intervals on 2- to 4-day-old epicotyl.

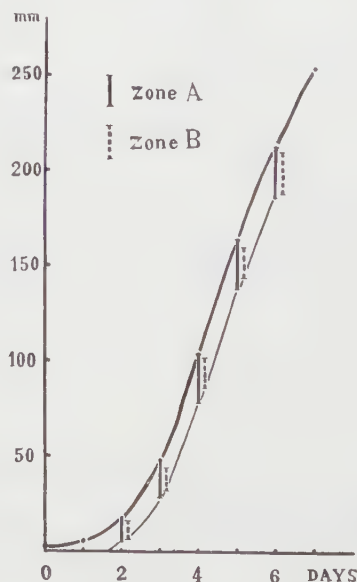


Fig. 3 Elongating region and $\frac{\partial^2 E}{\partial x^2}$ value of epicotyl.

Ordinate: Distance from the cotyledonary node towards shoot apex.

Zone A: Elongating region of epicotyl.

Zone B: Region showing positive value of $\frac{\partial^2 E}{\partial x^2}$.

Curves are drawn through both upper and lower ends of the elongating regions.

Here we point out that during earlier stages of germination, cotyledon has a higher potential level as compared with other parts of the embryo, i.e., in 2- or 3-day-old embryo the potential gradient between the cotyledon and epicotyl part is first steep, gradually becoming a gentle slope in later germination stage (Fig. 5).

Evidence of the fact that cotyledon in earlier stages of germination has a higher potential level has already been given for other plants, e.g. for *Helianthus annuus*, *Pisum sativum* by Kinoshita⁷⁾ and in *Vigna sesquipedalis* by one of us^{2,6)}. And the lowering of the positive potential of cotyledon in later germination stages was observed in *Vigna*⁶⁾ too.

According to the data reported by Ramshorn⁸⁾ and Oda¹⁾, the apical part of hypocotyl in hypogeal plants was shown as more positive. Although they did not measure the potential of cotyledon itself, their results seem to suggest a high positive potential of cotyledon.

Assuming potential difference to be due to spatial difference in ionic concentrations, it is reasonable to consider that there should be a remarkable gradient of ionic concentration between cotyledon and axial part of seedling in the first stages of germination. In fact, it was indicated in another experiment using *Vigna* embryo⁶⁾ that the ratio of ionic concentration in both parts, a ratio first fairly large, decreases with growth of embryo because of the transport of inorganic ions and metabolites from cotyledon to axial part of seedling.

Although, as yet, we have no information on the chemical composition of the embryo of *Phaseolus angularis*, we have some data to support the above conclusions. In Fig. 4 we give the ratio of cotyledon dry weight (*DW*) to fresh weight (*FW*) which decreases in linear relations to progressing culture age.

Here we can also examine the validity of the presumed relation between electric potential and ionic concentration²⁾ in cotyledon. In Fig. 5, besides the observed values of potential (c. f. METHOD), the theoretical curve of the following half-empirical equation is plotted.

$$E_c = \frac{RT}{F} \log \frac{DW}{FW-DW} + 50 \text{ mV}$$

In this equation, E_c means electric potential of cotyledon, the constant 50 mV is empirically introduced, and the variable $DW/FW-DW$ is to be expected to change approximately in proportion to ionic concentration in cotyledon. The observed values of FW and DW shown in Fig. 4 were inserted into this formula. Although the observed values of E_c are somewhat dispersed, the parallelism between them and the theoretical values cannot be regarded as insignificant.

The kinds of ions which are mainly responsible for maintenance of the electric potential, are yet to be clarified.

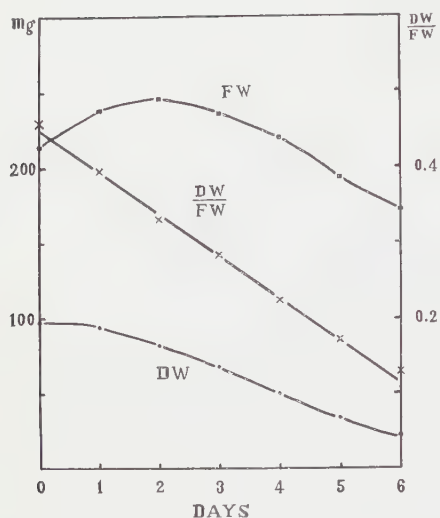


Fig. 4. Concentration change of tissue substances in cotyledon.

FW: Fresh weight of cotyledon per embryo.

DW: Dry weight of cotyledon per embryo.

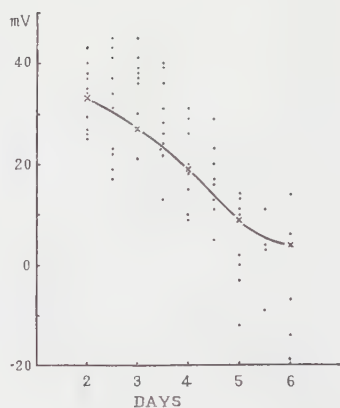


Fig. 5 Change of potential difference between cotyledon and cotyledonary node with growth of the plant.

● (dots): Observed values.

—x— : Theoretical curve

(see text).

The cotyledon of 5- to 6-day-old embryo shows a lower potential level than the level of the part of epicotyl in which elongation had already stopped (Figs. 1-D and E). This seems to support the consideration that, in the axial organ, a transformation in the metabolic pattern of the anabolic into a catabolic may occur in an epicotyl of *Phaseolus*, a consideration suggested by Oota et al. for the hypocotyl of *Vigna sesquipedalis*^{4,5)}, and in this respect, the part of the epicotyl in which elongation has ceased may become a storage organ, showing in later germination stages a higher potential level than other parts of embryo.

Summary

1. Determination was carried out for distribution of electric potential on the surface of a typical hypogeal embryo, *Phaseolus angularis*, and distribution curves of the potential were obtained for various stages of germination up to 6 days after sowing.

2. A valley of potential curve appears in the elongating region of epicotyl of 2- to 6-day-old embryo, and the hypothesis for the epigeal plant given in the former paper²⁾, viz. the hypothesis that distribution of elongation velocity along the elongation axis coincides with distribution of second spatial derivative of the potential with respect to the elongation axis, seems to be applicable to the epicotyl of *Phaseolus angularis*.

3. Cotyledon has a higher potential level than the elongating part of epicotyl and the potential gradient between cotyledon and epicotyl is steep in the earlier

stages of germination, gradually decreasing in later stages.

The writers express their gratitude to Prof. T. Mori for his helpful advice and discussions.

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Auxin Action and Pectic Enzyme*

by Shizuko YODA**

依田静子:** オーキシン作用とペクチン質酵素

Received March 5, 1958

In the etiolated pea stem a remarkable parallelism has been found between the effect of auxin at various concentrations on the water absorption and that on the pectin methylesterase (PME) activity.¹⁾ And it has been suggested that the activation of PME by auxin causes an increase in the water uptake stimulated by auxin. Some experiments were performed, in order to see if these two activities change in parallel with each other under the effects of antiauxins and metabolic inhibitors. This paper reports that the parallelism was observed in every case so far tested.

Material and methods

The third internode, 3.0 to 3.5 cm. in length, of seedlings of Alaska pea grown in the dark for 6 days at 25.5° was used as the material, as before¹⁾. The apical 5 mm. of the internode was discarded, and two 5 mm. pieces were excised from the adjacent part of the internode. About 1 g. of these pieces were floated at the surface of single and mixed solutions of IAA and various reagents. They were kept in the dark for 1.5 hours at 25.5° with aeration by bubbling. Water absorption was expressed by a percentile increase of fresh weight of these pieces.

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** Department of Botany, Faculty of Science, Kyoto University, Kyoto, Japan. 京都大学理学部植物学教室

PME activity was determined as the following.* Stem pieces were frozen and ground mixed with dry ice, and suspended in a 10% sodium chloride solution. This suspension was added to a pectin solution** and kept at $31 \pm 0.1^\circ$ for 1 hour. The increasing carboxyl due to demethylation was titrated, using methyl red as an indicator. The PME activity was expressed in μ moles of the methoxyl split off*** from 1 g. fresh weight of stem pieces.

Results

a) Treatment of living tissue.

The concentration of auxin optimal for both the water absorption and the PME activity of the pea stem was 10 mg./l. according to previous studies¹⁾. Hence auxin was used mainly in this concentration.

2,4-Dichloroaniline (DCA) is antagonistic to IAA in the effect on elongation and respiration of *Avena* coleoptile sections²⁾³⁾. Stem pieces were floated in combined solu-

tions of IAA (10 mg./l.) and various concentrations of DCA. The results are represented in Fig. 1. Both the water absorption and the PME activity were low in the absence of IAA, and DCA exerted little effect on them. On the other hand, the two activities enhanced by the presence of IAA were depressed by DCA.

As shown in Fig. 1, a remarkable parallelism was found between the water absorption and the PME activity, both in the presence and in the absence of IAA.

In Fig. 1, the way of setting ordinate values is simply empirical. But correspondence between the observed values of the two activities is so remarkable in any of the experiments as described previously¹⁾ and in this paper that the ordinate set may not be regarded as meaningless. The parallel-

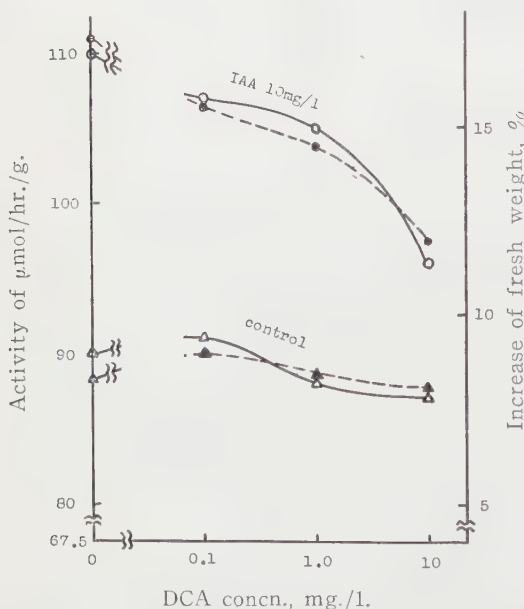


Fig. 1 Pectin methylesterase activity (—○—, —△—) and water absorption (---●---, ---▲---) of pea stem sections aerobically treated for 1.5 hours by various concentrations of DCA in the presence (upper curves: ○, ●) and in the absence (lower curves: △, ▲) of 10 mg./l. IAA.

*For further details, see Yoda¹⁾.

**Citrus pectin ($\text{CH}_3\text{O}=9.14\%$) was used in the present case, instead of apple pectin ($\text{CH}_3\text{O}=9.45\%$).

***For blank test, suspension of the tissue homogenate was heated on boiling water for 5 minutes, added to pectin solution, and titrated.

ism mentioned in the preceding and the present papers means that the two curves coincide with each other when the ordinates are put in this particular way.

Maleic hydrazide (MH) is also regarded as an auxin antagonist as it inhibits the auxin-induced growth and makes the tissue recover from the growth inhibition caused by supraoptimal concentrations of IAA^{4,5}). Four various concentrations of MH were combined with IAA solutions at four different concentrations, namely 0.1, 1.0, 10 and 100 mg./l. (Fig. 2). In the lowest concentration of IAA water absorption was inhibited by MH. On the other hand, the water absorption inhibited by the highest concentration of IAA was removed by MH. In any combination of concentrations of IAA and MH, including the cases of absence of either one, the PME activity corresponded well to the water absorption.

Coumarin is reported to be a growth inhibitor. And as the inhibition by it is removed by BAL, it is thought that coumarin inhibits SH^{6,7}). However, it is also reported to increase growth in the presence of small concentrations of auxin. Experimental results for coumarin are shown in Fig. 3. Coumarin promotes the water absorption best at 1.0 mg./l. in the presence of IAA and at 0.1 mg./l. in the absence of IAA, and it inhibits the water absorption at higher concentrations. Here again the PME activity changes just as the water absorption does.

p-Chloromercuribenzoate (PCMB) and monoiodoacetic acid are regarded as SH inhibitors. The curves of concentration effect of these two substances are shown in Figs. 4 and 5. In each of these figures the curve of water absorption and that of the PME activity affected by the inhibitor are in parallel with each other.

Also with 2,4-dinitrophenol (DNP), which is regarded as an energy uncoupling agent, the water absorption and the PME activity are inhibited at high concentrations of DNP (Fig. 6). In this case, the former is inhibited more than the latter if the same ordinates are adopted as in other cases.

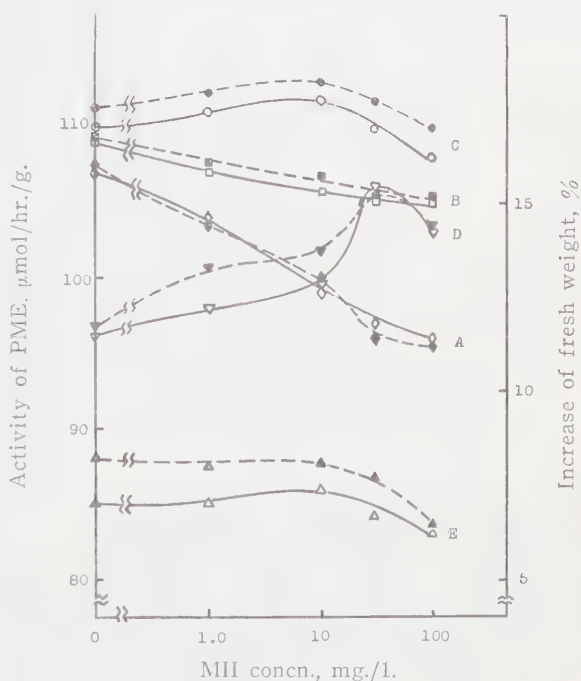


Fig. 2 Pectin methylesterase activity (\circ , \square , \diamond , ∇ , \triangle) and water absorption (\bullet , \blacksquare , \blacklozenge , \blacktriangledown , \blacktriangle) of pea stem sections aerobically treated for 1.5 hours by various concentrations of maleic hydrazide in the presence of 0.1, 1, 10 and 100 mg./l. IAA (pairs of curves, A, B, C and D, respectively) and in its absence (E).

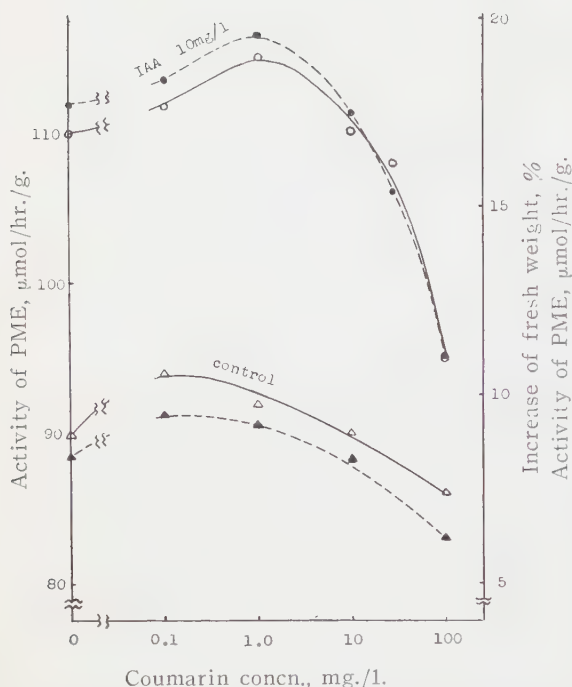


Fig. 3. Pectin methylesterase activity (—○—, —△—) and water absorption (---●---, ---▲---) of pea stem sections aerobically treated for 1.5 hours by various concentrations of coumarin in the presence (upper curves: ○, ●) and in the absence (lower curves: △, ▲) of 10 mg./l. IAA.

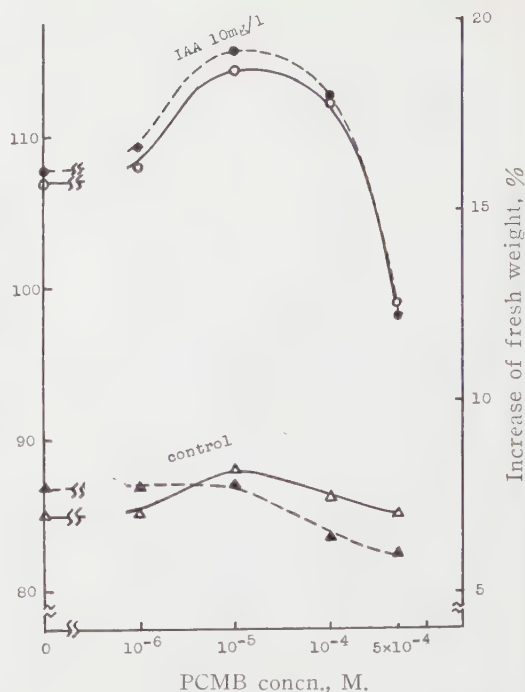


Fig. 4. Pectin methylesterase activity (—○—, —△—) and water absorption (---●---, ---▲---) of pea stem sections aerobically treated for 1.5 hours by various concentrations of p-chloromercuribenzoate in the presence (upper curves: ○, ●) and in the absence (lower curves: △, ▲) of 10 mg./l. IAA.

b) Effect of substances *in vitro*.

In the above-mentioned cases, the PME activity was measured with the homogenates obtained from the living tissues which were treated by the various substances. In order to see if the observed effects of those substances were due to their direct effects on the enzyme activity, those substances were added to the homogenate of the pea stem pieces which had, or had not, been treated by IAA alone. The substances mentioned above and phenylacetic acid, said to be a semiauxin, were added to the tissue homogenates, and the PME activity was measured. The results are represented in table 1. The PME activity was significantly increased by the SH inhibitors, namely PCMB, monoiodoacetic acid and coumarin, in their higher concentrations used. Similar results were obtained with DCA, which may also react with thiol group, according to Leopold.⁸⁾ When no such substances were added, the PME activity of homogenate of the tissue which had been pretreated with IAA was higher than that of the tissue without the pretreatment. Consequently, the effects of the

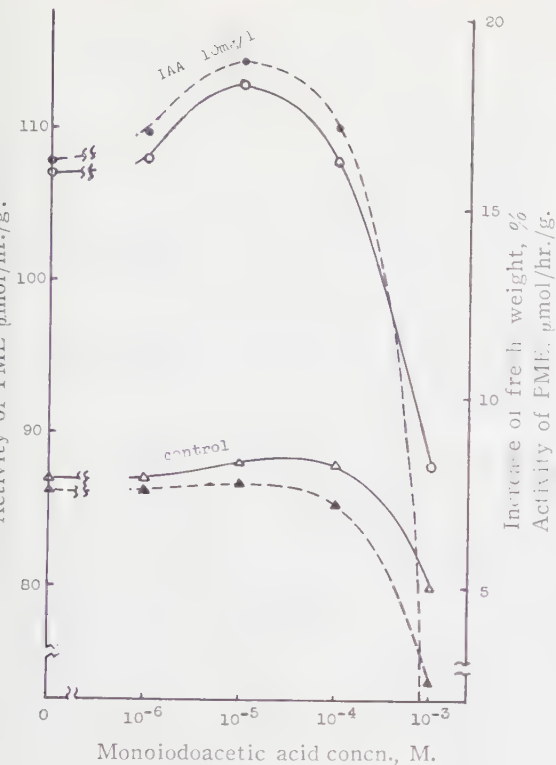


Fig 5 Pectin methylesterase activity (—○—, —△—) and water absorption (---●---, ---▲---) of pea stem sections aerobically treated for 1.5 hours by various concentrations of monoiodoacetic acid in the presence (upper curves:○, ●) and in the absence (lower curves:△, ▲) of 10 mg./l. IAA.

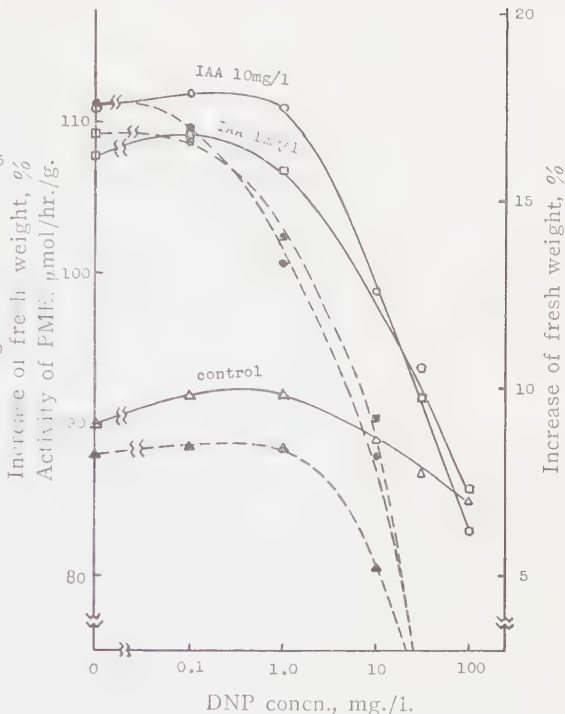


Fig. 6. Pectin methylesterase activity (—○—, —□—, —△—) and water absorption (---●---, ---■---, ---▲---) of pea stem sections aerobically treated for 1.5 hours by various concentrations of 2,4-dinitrophenol in the presence of 1 and 10 mg./l. IAA (rectangles and circles, respectively) and in its absence (triangles).

Table 1. Direct effect of various agents on the pectin methylesterase activity of homogenate of pea stem sections with and without pretreatment by IAA.

Substance and its concentration		Tissue pretreatment			
		None		IAA 10mg./l.	
		$\mu\text{mol/hr./g.}$	Diff. from control	$\mu\text{mol/hr./g.}$	Diff. from control
None (control)		87	0	110	0
DCA	1 mg./l.	96	+ 9	114	+ 4
	10 "	111	+24	122	+12
MH	1 "	90	+ 3	108	- 2
	10 "	87	0	112	+ 2
Coumarin	10 "	104	+17	—	—
	100 "	112	+25	121	+11
PCMB	10 ⁻⁵ M.	121	+34	129	+19
	10 ⁻¹ "	100	- 73	105	+ 55
Monoiodoacetic acid	10 ⁻³ "	90	+ 3	109	- 1
	10 ⁻² "	137	+50	143	+33
DNP	1 mg./l.	89	+ 2	109	- 1
	10 "	84	- 3	102	- 8
Phenylacetic acid	1 "	87	0	—	—
	10 "	86	- 1	—	—

substances were less obvious with the former homogenate than with the latter homogenate. DNP, MH and phenylacetic acid did not significantly influence the PME activity whether stem sections were previously treated by IAA or not.

In order to check if PCMB, monoiodoacetic acid and coumarin increased the titration value either of the pectin solution or of the tissue homogenate, those substances were added to each of the latter. But no significant changes in the titration values were observed. Therefore the SH inhibitors used must have increased the PME activity.

At any rate, it may be concluded that the substances so far tested, including IAA, exert effects on the PME activity of the living tissue in manners quite different from the direct ones on the tissue homogenate.

Discussion

It was demonstrated above that the water absorption of pea stem sections was affected by any of the various substances used, quite in the same way as the PME activity, when measured with the homogenate of the treated tissue. These results are similar to those obtained by treatment with IAA in various concentration¹⁾. Such a remarkable coincidence in so many cases cannot be incidental. It was also confirmed in the preceding¹⁾ and the present papers that the effect of those substances are not due to their direct effect on PME.

For causal relation between PME activity and water absorption, the following two possibilities can be suggested. 1) Auxin and the substances experimented affect the PME activity of cells, and this in turn affects the water absorption of the cells by changing the extensibility of cell walls (if the reverse sequence is impossible); and 2) There is no causal sequences between the PME action and the water absorption, but these are affected in parallel with each other by the common cause.

The first alternative is proposed, because the extensibility of the wall of young growing cells could be affected by some enzymes acting on pectic substances, which are reported to constitute the continuous cementing phase of the wall of such cells. However, it should be demonstrated that PME actually increases the extensibility of the cell wall.

The second alternative seems to be less probable because of the remarkable parallelism between the two activities in so many different cases. The fact that DNP, an energy uncoupler, inhibited the water absorption more severely than the PME activity may be rather favourable for the first alternative, because, even if the wall has become more extensible, active water absorption can not proceed when sufficient energy is not available for it.

Summary

Young pea stem sections were treated by 2,4-dichloroanisole (DCA), maleic hydrazide (MH), coumarin, p-chloromercuribenzoate (PCMB), monoiodoacetic acid and 2, 4-

dinitrophenol (DNP), in combination with IAA or without it.

Promotion and inhibition of water absorption due to various concentrations of those substances were amazingly paralleled by pectin methylesterase (PME) activity of the homogenate of the treated tissue. Compared with other substances, DNP inhibited water absorption relatively more severely than PME activity.

The effects of substances on the PME activity in the case of tissue treatment were not due to their direct effects on the enzyme preparation.

A hypothesis is proposed that activated PME increases extensibility of walls of growing cells, and consequently their water absorption.

Acknowledgements

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Studies on Spore Germination of Hepaticae 4*

Makinoa crispata (Steph.) Miyake

by Hiroshi INOUE**

井上 浩:** 苔類の胞子発芽 (4) マキノゴケ*

Received March 31, 1958

Makinoa crispata was first described by Stephani (1897) under the genus *Pellia*, based on Faurie's collection made in Japan. Two years later, Miyake (1899) studied on the sexual organs and the sporophyte, which resulted in his proposal of new genus, *Makinoa*, for this species. *Makinoa* is a famous genus for its remarkably large spermatozoid in the Bryophyte (Miyake 1899), and Nakai (1943) proposed the family Makinoaceae for this genus. Before Nakai, this genus had been included in the Dilaenaceae (Verdoorn 1932) or the Pallaviciniaceae (Evans 1939). Since Nakai's proposal of the family, Japanese bryologists have adopted it, but Müller (1951) placed the genus in the Dilaenaceae together with *Pallavicinia*, *Moerckia*, and *Calycularia*.

Makinoa comprises a single species, *M. crispata* (Steph.) Miyake, which has been known from Japan, Korea, Formosa and China (Hattori 1952). This species usually inhabits at much humid and more or less shady sites. The sporophytes appear from March to April. The present study was undertaken in order to determine the pattern of the sporeling of this species.

The spores for this study were collected at Yoshino-mura of Kochi Pref. (Mar. 24, 1955) and Kitago-mura of Miyazaki Pref. (Mar. 30, 1957). The spores were sown 10 days after the collection. The media for germination were used (1) Knop's solution, (2) Knop's agar (2%), (3) Meyer's solution, (4) sterilized *Sphagnum* mats moistened with Knop's solution. In any case the germination took place in 10 days after seedling, but except for the *Sphagnum* mats the development after 3-celled stage was mostly suppressed and the sporeling died. The complete pattern was observed in the *Sphagnum* mats.

Germination

The spores are spherical measuring 20–32 (mostly 24–27) μ , and are light to blackish brown with many reticulated ridges on the surface. The spore coat is uniformly thickened.¹⁾ In the endospore there are many chloroplasts and few oil-drops which

* Continued from J. Jap. Bot. 33: 6 (1958).

** Botanical Institute, Faculty of Science, Tokyo University of Education, Ohtsuka, Tokyo. 東京教育大学理学部植物学教室

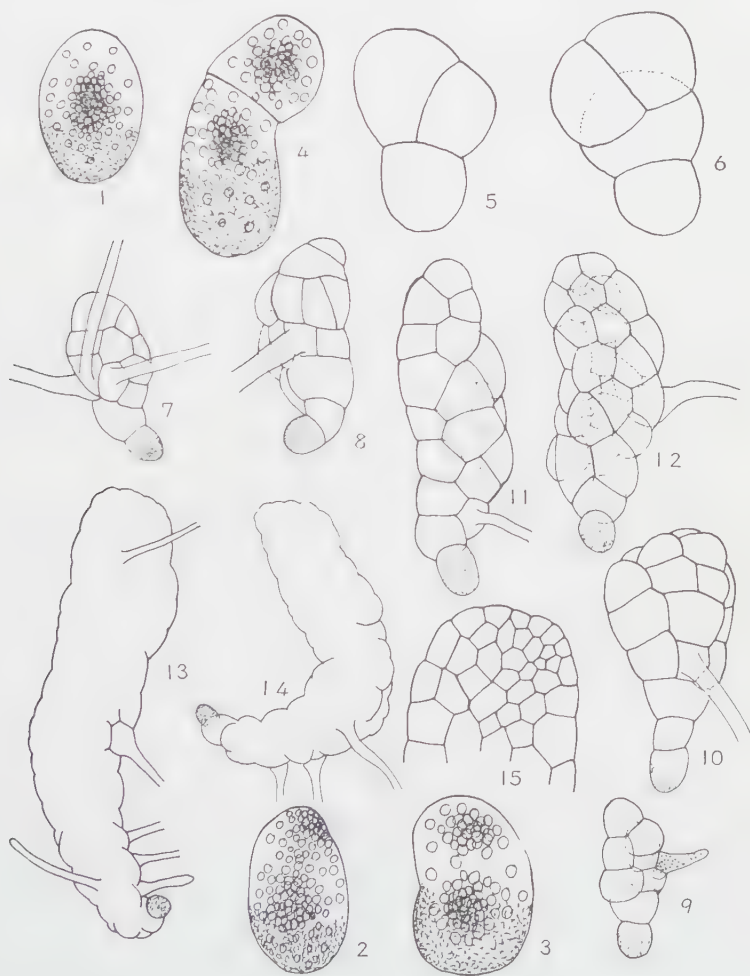
¹⁾ Miyake (1899) gave the measurement of the spore as 20–25 μ , and Horikawa (1929) as 25.5–28 μ . The spore measurement is more variable, even in a single sporangium, as shown in this paper. Miyake's description on the spore coat, as "much thicker in one side than in the other half", seems to be that of died spore, in which the endospore is much shrunk and the spore coat, by surface view, seems to be irregularly thickened. But this is abnormal feature caused by the spore death.

are faintly stained by Sudan III. The chloroplasts are usually crowded surrounding the comparatively large nucleus (so that, by the surface view, there seems a green mass in the center of the endospore).

Before the germination, the chloroplasts disperse and the oil-drops mostly disappear. The spore much swells and elongates, so that the spore coat ruptures before the formation of the first wall (Figs. 1-3). Before the first transverse wall is formed, the nuclear division takes place; then, two crowded masses of the chloroplasts are observed in the germ cell (Fig. 3). The similar feature of the chloroplasts is observed also in the succeeding cell divisions (at least in earlier stage).

The first wall separates the germ cell into two unequal cells (Fig. 4).

The smaller cell grows nearly as large as, or a little larger than, the other (basal cell) and is divided with transverse wall or occasionally with longitudinal



Figs. 1-15. Various stages of sporeling in *Makinoa crispata* (Steph.) Miyake. For explanation see text, Figs. 1-6, $\times 360$; 7-12, $\times 160$; 13-14, $\times 96$; 15, $\times 160$.

one (Figs. 5, 6). The basal cell does not make further growth and division as well, and has brown spore coat throughout the developmental course of the sporeling. The pattern of the development may be somewhat modified by outer conditions. But, in any case, the apical cell with two cutting faces is formed before the 10-celled stage. (Figs. 7-9).

The first appearance of the rhizoid (Fig. 9) does not occur before about 6-celled stage (but in very few cases it was observed at the 4-celled stage). The rhizoid initial cell is usually a little smaller and contains fewer chloroplasts than the other cells. The wall of the rhizoid, at first, is faintly brownish. However, it soon becomes purplish brown. So, when rhizoids well developed, it is much embarrassed whether or not there is distinct wall between the rhizoid and its initial cell: the rhizoids are always continuous from their initial cells. The similar rhizoids are formed at random, but, when the dorsi-ventrality of the sporeling is confirmed, the rhizoids arise only from the ventral side (Figs. 13, 14). The chloroplasts in the rhizoid disappear.

The development of the thallus is caused by the activity of the apical cell with two cutting faces. The apical cell, however, becomes indistinct by the formation of meristematic apical cells (Fig. 15). The thallus formed by the meristematic cells becomes 4-7 cells thick in the central portion where the midrib of the thallus may develop.

The oil-bodies, which are not stained by Sudan III, are formed in the cells at about the 10-celled stage (but occasionally observed also in earlier stage). They are much small and spherical (about 1μ), about 10 in number per cell, and white in color. They are evidently the same with those of the adult plant except for their size.

Discussion and Conclusion

The characteristic features in the sporeling *Makinoa crispata* are (1) the first cell division which takes place after the expansion of the exospore, (2) the basal cell which is always distinct and does not develop further, (3) the much crowded chloroplasts surrounding the large nucleus, (4) the deep brown rhizoid which is in direct continuation from the basal cell of rhizoid and appears after the 4-celled stage, at least, (5) the development of the young thallus which is derived from the apical cell with two cutting faces, and (6) the apical cells of the young thallus, which form the meristematic apical region.

Schiffner (1901) and Horikawa (1929) discussed the systematic position of *Makinoa* and the latter author stated the close relation to the Metzgerioideae and the Leptothecaceae (Pallaviciniaceae) after the extensive studies of the plant. The related genera whose sporeling pattern were studied are *Metzgeria* (Goebel 1915, 1930), *Pallavicinia* (Haupt 1918, Wolcott 1942, Kachroo 1956), and *Moerckia* (Mader 1929). In those genera, except for *Metzgeria*, the first cell division may take place within the exospore and before the expansion of the exospore several cells are formed.

In *Metzgeria* the first wall is formed after the rupture of the exospore as *Makinoa*, but the protonema is filamentous. Compared with them, *Makinoa* has a distinct pattern of the sporeling. Another related genus to *Makinoa* is *Calycularia*, whose literature is not seen, and which may have also another pattern (unpublished data); that is, the first cell division takes place within the exospore.

Goebel (1930) included *Makinoa* in the 'Aneuraceen'. The sporeling pattern in *Riccardia* (*Aneura*) was studied by Leitgeb (1874-1882), Showalter (1925), and Goebel (1930). According to their studies, the exospore ruptures before the formation of the first wall by the elongation of the endospore as in *Makinoa*. However, the earlier stage of the development in *Riccardia* takes much different course, and filamentous protonema develops and the apical cell has two cutting faces as in *Makinoa* (Campbel 1905). The pattern in *Metzgeria* is allied to that in *Riccardia*.

Between the Pelliacae and the Makinoaceae are some essential distinctions in the sexual organs and the sporophytes (Miyake 1899). Besides those differences, the sporeling pattern of the Pelliacae (Wolfson 1928, Goebel 1930) and of the Makinoaceae are remarkably different.

Summary

The sporeling pattern of *Makinoa crispata* is described and discussed. The first cell division takes place after the rupture of the exospore; the second or third division is longitudinal and the sporeling develops into several-celled primary plant whose apical cell has two cutting faces; the apical cell forms the meristematic apical region which becomes 4-7 cells thick at middle; the first rhizoid develops normally after the 6-celled stage and is characteristically purplish brown as that of the adult thallus; the oil-bodies in the earlier stage of development are similar to those of adult thallus except for their size. This pattern may be named as *Makinoa*-type. The relationship between the Makinoaceae and other related families is discussed from the view point of the sporeling pattern.

The writer is much indebted to Dr. S. Hattori of the Hattori Botanical Laboratory and to Prof. H. Ito of Tokyo University of Education for their very suggestive advice.

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花粉の生理, 形態学的研究

第7報. 花粉の発芽におよぼす各種アミノ酸の影響について*

沢田 義 康**

YOSHIYASU SAWADA**: Physiological and Morphological Studies on the Pollen Grain.

Part 7. On the Effects of Some Amino Acids on the Germination of the Pollen Grain and on the Growth of the Pollen Tube.

1958年2月3日受付

花粉は, sucrose agar 培地上で発芽が容易な種類ときわめて発芽が困難な種類とに大別される。しかし sucrose agar 培地上で発芽が困難な花粉でも, 適量のアミノ酸をくわえた sucrose agar 培地上では, 容易に発芽することがみいだされた^{3,4)}。本報は sucrose agar 培地上ではほとんど発芽しない *Paris hexaphylla* Chamisso. の花粉とその柱頭, 子房, ならびに sucrose agar 培地上で発芽が容易な *Oryza sativa* L.⁵⁾ の花粉とその雌ずい中に含まれる主要なアミノ酸の種類をクロマトグラフ法によって調べるとともに, これら組織中に含まれるアミノ酸を, sucrose agar 培地に添加して花粉を培養する場合, 両種花粉の発芽, および花粉管の伸長と, 添加したアミノ酸の種類との間に一・二の興味ある関係のあることをしたので, ここに報告する。

供試材料ならびに実験方法

(1) 供試材料

sucrose agar 培地上での花粉の発芽習性上興味ある対立をなす種類である *Paris hexaphylla* Chamisso. (クルマバツクバネソウ) と *Oryza sativa* L. (水稻) の一品種「富錦」を材料植物としてえらび, いずれも蒴裂開直後の花粉と, 雌ずいを材料とした。

(2) 測定方法

まず予備実験により, 花粉の発芽培地としての最適 sucrose 濃度は, *Paris hexaphylla* Chamisso. では 2%, *Oryza sativa* L. 富錦では 12% であり, 最適 agar 濃度は, いずれも 1%, pH は 6.5 であることをたしかめたので, 本実験を通じてこの条件でそれぞれ 0.0005%, 0.001%, 0.003%, アミノ酸添加培地をつくり, これに花粉を散布した。*Paris hexaphylla* Chamisso. は 25° で4時間, *Oryza sativa* L. 富錦では 25°~28° で15分間培養し, 花粉 100 個体の発芽率をもとめ, 30 個体以上の花粉管長をマイクロメーターで測定した。control は基本培地をもちい, アミノ酸をくわえた培地の場合と同様な条件下でおいおいの発芽率と花粉管の長さを測定した。なお使用したアミノ酸は, 味の素製薬株式会社製のもので, 次の 19 種類である。

Alanine, arginine, aspartic acid, cysteine, cystine, glutamic acid, glycine, histidine, hydroxyproline, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan および valine. 他方花粉, および雌ずい中に含まれるアミノ酸の種類については, *Paris hexaphylla* Chamisso. では花粉 0.2g, 柱頭 0.2g, 子房 0.5g, を材料とし, また *Oryza sativa* L. (富錦) では花粉 0.1g, 雌ずい 0.2g を材料としてバーバー・クロマトグラフ法^{D)}によりアミノ酸の種類をしらべた。

* 本研究の要旨は昭和 32 年 10 月 第 22 回日本植物学会大会に発表

** 北海道学芸大学旭川分校生物学教室 Division of Biology, Asahikawa Branch, Hokkaido Gaku-gei University, Hokkaido, Japan.

spot の大きさ、発色の程度によって、卅，卅，
十，十，一，の5段階の符号をもってその含量の
比較値を表示した。ただし，一はアミノ酸に比べ
ぜんみとめられないことをしめす。展開剤として
は，一次元紙クロマトグラフでは butanol-acetic
acid-water (4:1:1)，二次元法では含水 phenol
(85%)，および butanol-acetic acid-water (4:
1:1) の両溶媒を併用した。展開は常法により，
それぞれ 40×2 cm，および 40×40 cm の No. 50
のろ紙を 25° で静置，乾燥後ニンヒドリンの

で最高の価をしめし，ついで aspartic acid，
histidine，cystein の順であった。つぎにアミ
ノ酸濃度を 0.001% に高めると，前述の 0.0005%
添加で良好な発芽がみられた種類のアミノ酸は，
さらに高い発芽率をしめした。またこの濃度では
じめて発芽促進がみとめられた種類のアミノ酸と
しては，alanine，glycine，leucine，threonine，
tyrosine，tryptophan および valine などがあ
げられる。さらに 0.003% までアミノ酸の濃度
をあげると，aspartic acid をのぞき，0.001%

Table 1. The effects of amino acids on the germination rate of the
pollen grain in *Paris hexaphylla* Chamisso.

Sorts of amino acids	Concentration of amino acid		
	0.0005%	0.001%	0.003%
	Germination rate	Germination rate	Germination rate
Control	0 %	0 %	0 %
D. L-Alanine	0	20.0	0
L-Arginine	0	0	0
L-Aspartic acid	42.7	56.1	68.8
L-Cysteine	34.4	88.1	0
L-Cystine	trace	0	0
L-Glutamic acid	81.0	85.5	64.3
Glycine	0	10.5	0
L-Histidine	34.8	71.6	12.9
L-Hydroxyproline	0	trace	trace
L-Leucine	0	6.2	trace
L-Lysine	0	trace	0
L-Methionine	trace	trace	0
L-Phenylalanine	0	0	0
L-Proline	10.6	30.5	trace
D. L-Serine	0	0	0
D. L-Threonine	0	7.8	0
L-Tyrosine	0	20.5	0
D. L-Tryptophan	0	24.8	0
D. L-Valine	0	23.0	0

0.02% ブタノール溶液を発色剤としてもちい，
アミノ酸の種類をしらべた。

実験結果および考察

(1) 花粉の発芽におよぼすアミノ酸の影響
0.0005% アミノ酸添加 sucrose agar 培地上
における *Paris hexaphylla* Chamisso. の花粉の
発芽率は (第1表参照)，glutamic acid 添加

濃度で顕著な発芽促進がみとめられた histidine，
glutamic acid では，かえって発芽率の減退が
みとめられた。

つぎに *Oryza sativa* L. の花粉についていうと，
(第2表参照)，0.0005% の arginine，alanine，
valine 添加した培地上で対照区に比しそれぞれ
いちじるしい発芽の促進がみられた。

さらに 0.001% アミノ酸添加培地では valine

Table 2. The effects of amino acids on the germination rate of the pollen grain in *Oryza sativa* L.

Sorts of amino acids	Concentration of amino acid		
	0.0005%	0.001%	0.003%
	Germination rate	Germination rate	Germination rate
Control	58.6 %	58.6 %	58.6 %
D. L-Alanine	73.2	75.0	81.8
L-Arginine	78.5	76.0	42.8
L-Aspartic acid	17.5	17.9	0
L-Cysteine	23.4	trace	0
L-Cystine	58.5	60.9	34.6
L-Glutamic acid	40.0	0	0
Glycine	63.5	54.1	52.5
L-Histidine	55.5	41.0	trace
L-Hydroxyproline	58.0	57.7	76.4
L-Leucine	50.0	68.7	52.7
L-Lysine	52.0	53.1	55.5
L-Methionine	45.6	45.3	45.7
L-Phenylalanine	60.0	78.4	55.5
L-Proline	52.2	57.5	72.1
D. L-Serine	50.5	72.8	81.8
D. L-Threonine	53.0	40.7	26.0
L-Tyrosine	50.0	29.6	0
D. L-Tryptophan	62.4	61.0	37.5
D. L-Valine	71.4	82.5	20.0

による促進が最も多いといえるが、ついで phenylalanine, arginine, alanine の順をしめし、0.0005% アミノ酸添加培地に比しいずれも発芽率の増加がみられた。また 0.003% アミノ酸添加培地では、alanine, serine の添加による発芽率がいちばん高く、他方 hydroxyproline, proline の場合にも発芽率の増加がみられた。しかし 0.001% アミノ酸添加により高い発芽率がみられた phenylalanine, valine は、0.0003% 濃度では急激に発芽の低下をきたした。またこの他のアミノ酸添加培地上の花粉の発芽は、control に比しいちじるしくわるい。

(2) 花粉管の伸長におよぼすアミノ酸の影響

Paris hexaphylla Chamisso. の花粉管伸長とアミノ酸との関係についてみると、第1表と第3表とを比較すればあきらかなように、0.0005%、0.001%ならびに 0.003% の各アミノ酸添加培地

を通じいずれも花粉の発芽を促進する作用をもつアミノ酸の添加により、この植物の花粉管の伸長も同様に促進される。しかもアミノ酸の種類、濃度と花粉管伸長促進との間の関係は、花粉発芽の場合とさきわめてよく一致する。

(3) 花粉に含まれるアミノ酸の種類

まず *Paris hexaphylla* Chamisso. の花粉に含まれるアミノ酸については、第4表にしめすように、glutamic acid がもっとも多く、histidine, aspartic acid, alanine がこれについて多く、その他、proline, tryptophan, leucine, cysteine なども多少ながらみとめられた。他方 *Oryza sativa* L. の花粉では、(第5表参照)、serine が最も多く、その他 alanine, tryptophan, arginine, hydroxyproline, phenylalanine, valine などが認められた。このように花粉に含

Table 3. The effects of amino acids on the growth of the pollen tube in *Paris hexaphylla* Chamisso.
(The numbers in each columns show the lengths of pollen tubes expressed by the micrometer scale)

Sorts of amino acids	Concentration of amino acid					
	0.0005%		0.001%		0.003%	
	$M^*\pm\delta$	V^{**}	$M^*\pm\delta$	V^{**}	$M^*\pm\delta$	V^{**}
Control						
D. L-Alanine			7.4 \pm 1.4	18.8		
L-Aspartic acid	10.2 \pm 2.5	24.5	14.5 \pm 3.2	22.0	15.9 \pm 2.1	13.2
L-Cysteine	7.7 \pm 1.3	16.8	13.4 \pm 3.6	26.8		
L-Cystine						
L-Glutamic acid	12.8 \pm 2.9	22.6	15.6 \pm 2.1	14.1	9.3 \pm 2.6	27.9
Glycine			6.2 \pm 1.5	24.1		
L-Histidine	8.9 \pm 2.1	23.5	17.5 \pm 3.7	21.1	10.2 \pm 5.1	50.0
L-Hydroxyproline						
L-Leucine			6.5 \pm 1.7	26.0		
L-Lysine						
L-Methionine						
L-Phenylalanine						
L-Proline	6.2 \pm 1.2	19.3	8.7 \pm 1.8	20.6		
D. L-Serine						
D. L-Threonine			7.1 \pm 1.9	26.5		
L-Tyrosine			8.5 \pm 1.3	15.2		
D. L-Tryptophan			8.6 \pm 1.7	19.7		
D. L-Valine			8.0 \pm 1.5	18.7		

* Mean

** Coefficient of variability calculated from $V=\delta/M\times100$

Table 4. The amino acids which were identified to be contained in the pollen grain and in the pistil of *Paris hexaphylla* Chamisso.

Sorts of amino acids	Stamen	Pistil	
	Pollen grain	Stigma	Ovary
D. L-Alanine	+	+	+++
L-Arginine	—	—	—
L-Aspartic acid	+++	++++	+++
L-Cysteine	±	±	+
L-Cystine	—	—	—
L-Glutamic acid	++++	+++	++
Glycine	—	—	—
L-Histidine	+++	+++	++
L-Hydroxyproline	—	—	+
L-Leucine	±	+	+
L-Lysine	—	—	—

Table 4. Continued

Sorts of amino acids	Stamen	Pistil	
	Pollen grain	Stigma	Ovary
L-Methionine	—	—	++
L-Phenylalanine	—	—	—
L-Proline	±	±	+
D, L-Serine	—	—	—
D, L-Threonine	—	—	—
L-Tyrosine	—	—	—
D, L-Tryptophan	±	++	++
D, L-Valine	—	—	—

Table 5. The amino acids which were identified to be contained in the pollen grain and in the pistil of *Oryza sativa* L.

Sorts of amino acids	Stamen Pollen grain	Pistil Stigma, Ovary
D, L-Alanine	++	+
L-Arginine	+	+
L-Aspartic acid	—	—
L-Cysteine	—	—
L-Cystine	—	—
L-Glutamic acid	—	—
Glycine	—	—
L-Histidine	—	—
L-Hydroxyproline	+	+
L-Leucine	—	—
L-Lysine	—	—
L-Methionine	—	—
L-Phenylalanine	±	—
L-Proline	+	±
D, L-Serine	+++	++
D, L-Threonine	—	—
L-Tyrosine	—	—
D, L-Tryptophan	+	+
D, L-Valine	±	—

まれるアミノ酸の種類は、植物の種類によってこととなるが、とくに興味のあることは、花粉粒そのものに大量に含まれている種類のアミノ酸の多くが、それを sucrose agar 培地にくわえると花粉の発芽を促進することである。しかし *Paris hexaphylla* Chamisso. の leucine, *Oryza sativa* L. の glutamic acid のように、花粉には含まれているが、培地にくわえても促進的影響をあたえない種類のアミノ酸もみとめられたが、この場合

は花粉そのものに含まれている量だけで、花粉の発芽にはじゅうぶんとみるべきであろう。

(4) 雌ずいに含まれるアミノ酸の種類

まず *Paris hexaphylla* Chamisso. の柱頭についてみると、(第4表参照), aspartic acid, glutamic acid, histidine がいずれも大量に含まれ、その他 tryptophan, leucine, alanine, がこれにつぎ, cysteine, proline もわずかながら

みとめられた。つぎに子房に含まれるおもなアミノ酸としては, aspartic acid, alanine がもっとも多く, ついで histidine, glutamic acid, tryptophan, methionine で, その他 hydroxyproline, cysteine, leucine, proline などがみとめられた。花粉, ならびに柱頭に大量に含まれている aspartic acid, histidine, glutamic acid などを sucrose agar 培地に添加することによって, 花粉の発芽および柱頭の伸長がいちじるしく促進されるが, このことから柱頭上での花粉の発芽に対して, これらのアミノ酸が密接な関係をもつことが予想される。一方, 柱頭にはみられなかった hydroxyproline, methionine, の存在がみられたが, この種のアミノ酸を sucrose agar 培地にくわえても, 花粉の発芽には影響がみられなかった。

つぎに *Oryza sativa* L. の雌ずいでは (第5表参照), 花粉の場合と同様に serine がもっとも多く, その他 alanine, arginine, hydroxyproline, tryptophan がみられた。しかも *Paris hexaphylla* Chamisso. の場合と同様に, 雌ずいに含まれるこれらの各種のアミノ酸を sucrose agar 培地にくわえて *Oryza sativa* L. の花粉を培養すると, 発芽促進の傾向がみられた。

以上の結果を総合すると sucrose agar 培地上ではほとんど発芽のできない *Paris hexaphylla* Chamisso. の花粉でも, 花粉, 柱頭および子房の三部分に共通してみられる aspartic acid, glutamic acid, histidine のようなアミノ酸を sucrose agar 培地に適量添加することにより, 花粉の発芽でいちじるしく促進されることがたしかめられた。また sucrose agar 培地上で容易に発芽する *Oryza sativa* L. の花粉の場合も, 花粉, 雌ずいに多量に含まれる serine, alanine の添加により, 花粉の発芽がいちじるしく促進される。このことから, 花粉の発芽に影響をおよぼすアミノ酸の種類は, 植物の種をことにすることによりことなるものと考えられる。久保²⁾は *Oryza sativa* L. の花粉をゼラチン培地上で, 適当な吸水条件のもとで発芽させると, 高率の発芽をみたし報告しているが, このような発芽条件下では, ゼラチンの加水分解によって生ずるある種のアミノ酸が発芽に好影響をおよぼすことも予想にかたくない。

本研究にさいしいろいろと御指導をいただいた北海道大学農学部田川隆教授に深謝の意を表する。

Summary

1. The pollen grain of *Paris hexaphylla* Chamisso. does not germinate on the sugar agar medium. By adding aspartic and glutamic acids, histidine or cysteine to the control medium, the pollen grain is, however, enabled to germinate readily.

2. Although the pollen grain of *Oryza sativa* L. can germinate to a certain extent on the control medium, the germination was markedly stimulated when arginine, valine or alanine was added to the medium.

3. The kinds of amino acids which accelerate the germination of the pollen grain of *Paris hexaphylla* when they were added to the culture medium, were found to be present in the pollen grain itself and in the pistil of this plant. A similar relation was found to be true in the case of *Oryza sativa* L.

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植物細胞膜の微細構造の研究

I. 成熟した綿毛細胞膜の原繊維系について*

井 養 藏**

YOZO MITU**: Studies on the Fine Structure of Plant Cell Walls I.
On the Fibrillar System of Full Matured Cotton Hair.

1958 年 3 月 13 日受付

ま え が き

ワタ (*Gossypium barbadense* L.)¹⁾ の種皮にある毛は綿毛としてひろく知られており、その細胞膜の微細構造については、各方面の研究分野において多くの報告がだされている。

Barkley (1948) は、じゆうふんに成熟した綿毛の細胞膜には大別して 5 つの部分があることを模式的にしめた。第 1 層は第 1 次細胞膜による最外層、その内側すなわち第 2 層は第 2 次肥大生長層の外層、第 3 層は多数の層による中層、第 4 層は内部からの第 2 次肥大生長層の内層、そして第 5 層は細胞内に残留物を有する細胞壁である。各層は主として一方向に走る原繊維を構成要素としているという。また Mühlethaler (1949) は、綿毛の第 2 次肥大生長層はほぼ平行であることを電子顕微鏡像によって明らかにし、偏光顕微鏡下では、そのおのおのの軸に対する走向はわずかに S 字型をなしているという。

Roelofsen (1950) は、若い綿毛の生長細胞膜の第 1 次細胞膜には外膜と内膜とがあり、外膜はすべての原繊維が長軸に平行に、内膜は大多數の原繊維が長軸に対し直角な方向に走る構造であることを電子顕微鏡像でみだしている。さらに偏光による複屈折の研究から従来のもらせん構造説は訂正すべきであることを強調している。Frey-Wyssling, Mühlethaler (1950) および Roe-

lofsen (1951) はフィコミセスで、また Roelofsen (1951) はムラサキツユクサの雄ずいの毛で、若い細胞の第 1 次細胞膜には外膜として方向の一定しない繊維の中、内膜としては長軸に直角な走向をしめす原繊維の層があることを電子顕微鏡像でみだしている。このような微細構造は、細胞の伸長とあらたな原繊維のかん入にともなう 2 次的な原繊維走向のあらわれとして説明されている。

O'Kelley と Karr (1954) は、綿毛の生長細胞膜や、*Bignonia capreolata* L. の花粉管およびダイコン (White Icicle variety) の根毛で短い繊維素原繊維の単位をみだし、この伸長と連合によって小繊維がかたちづくられると説明している。Bailey et al. (1957) はアベナ子葉鞘の表皮細胞の第 1 次細胞膜をベクチナーゼおよびエタノールアミンで処理した材料をもちいて電子顕微鏡的に原繊維走向をしらべ、細胞の外膜は若いときは長軸方向に走る 10—15 層の原繊維層からなり、生長後のものでは 25 層から構成され、これらの原繊維層の先端はつぎつぎに一般型の第 1 次細胞膜に接着しているといっている。また彼らは、放射方向の細胞壁では長軸に直角な裁断線に向って走る原々繊維による原繊維層で構成されていることをみだしている。

原繊維系の構造を純粹にのぞくためには、原繊維間の充填物質をとりいぞくことが必要であっ

* Contributions from the Divisions of Cytology and of Genetics, Botanical Institute, Faculty of Science, University of Tokyo, No. 371.

** 東京大学理学部植物学教室 Botanical Institute, Faculty of Science, University of Tokyo, Tokyo, Japan

て、Weise, Peterson, Harlow (1939) によると木材をエタノールアミンで抽出するとよい結果が得られるといふ。この方法で抽出した原組織繊維の性質をしめし、Cross & Bevan セルローズと等しい値をしめしている。Afzelius, Erdtmann, Sjostrand (1954) は、ヒカゲノカズラの乾原組織を硫酸で、同時に亜硫酸をとり、塩素で漂白する方法をもちいている。

筆者は綿毛細胞膜縮微細構造に興味をもち、とくに細胞全体の形態と原組織系との関係を光学顕微鏡でしらべるために乾燥固定、切片、浸染における原組織の界面の問題および密集する原組織系を、なるべく簡単にみやすくすることを試み、従来諸研究にみられる多くの不一致点を解明したいと思つた。

材 料 と 方 法

材料：1953年に教室の畑に栽培したワタ*の種子に生じた毛をとり、種子はそのまま乾燥しきったもので、親木に着生したまま乾燥したものを採集した。栽培したワタの果実は1心室に6~7粒の種子よりなる1コの球を形成する。

方法 1. 綿毛の採取

1コの球に生じた綿毛を刈りとり、試験管に入れ、アセトンにじゅうぶんにくわえ、数時間後エーテルにとりかえ、さらに数時間ワックスの抽出をした。

方法 2. 充填物質の分解

原組織系の間げきを充填しているペクチンやヘミセルローズを分解し、アルカリによる溶解をしやすくするために次の溶液を準備した。

KCl 飽和水溶液	1 容積
ZnCl ₂ "	1 "
H ₂ SO ₄ d=約 1.53	1 "

はじめ材料に KCl 液をじゅうぶんに浸透し、次にべつの容器に上澄液をとり、これにくわえた KCl 液と等量の ZnCl₂ 液をくわえてよく混合したのを、材料にそそぐ。十分浸透したら H₂SO₄ の定濃度**のものを同様にべつの容器中で混合し

材料にそそぐ。10分放置したら、上澄液をとり、材料を水に入替する。このとき綿毛の表面に霜状にあらわれる結晶***は溶解しきる。

方法 3. 充填物質および残留細胞質の除去

上述の方法で充填物質は一部分分解されているのでさらに次の溶液をもちいて純粋にする。

無水モノエタノールアミン	1 容積
40% ヒドラジンヒドラート	"

この混合液中に10分夜放置し溶液を傾斜するかまた振りまわして溶液に一酸化あと、濃アモニア水で洗い、さらにじゅうぶん水洗する。この段階で原組織系はほぼ純粋になっていると考えられるが、外観には大きな変化はない。

方法 4. 綿毛の膨潤と洗滌

方法 1—3 では脱水状態で処理しているために膨潤その他による綿毛の変形はほとんど起きない。しかし綿毛を d=1.53 ぐらいの硫酸で処理するときむくみ丸状におじれる。これははじめに硫酸に接する表面ちかくがゲル化し硫酸の浸透をきまけるためにおこる構造上の局部的変化によるかと思つてゐる。これをなくするには硫酸に酢酸をくわえて使用する。もちいた混合液の処方は、

酢酸（氷酢酸）	1 容積
硫酸（定濃度）*	9 "

である。硫酸・酢酸液の濃度は検鏡調整を要する。方法 3 で処理した綿毛は、この溶液中で均等に膨潤する。同時に原組織間の残存充填物質は硫酸でよく分解される。じゅうぶん水洗したのちにヒドラジン・エタノールアミンで処理すると分解物を抽出する。濃アモニア水で洗滌したのちじゅうぶんな水洗をおこなう。

方法 5. 超音波処理による原組織系の拡張。

原組織の密集をほぐして原組織系をみやすくするために超音波にあてた。もちいた装置は島津製で、直径 20cm., 深さ 30cm. ぐらいのガラス製油槽の油面から 25cm. ぐらいの深さのところに水晶板を水平に固定してある。

電圧（高周波の）	1200 V.
水晶板の固有振動数	600~700 K.C.

* 種子はブラジル産でタキイ種苗 K.K. から購入したもの *Gossypium barbadense* L. にちかいものと思われる。

** あらかじめ H₂SO₄ d=1.53 ぐらいのものを調製し、時計皿に一滴づつとりこれに綿毛を投じ、膨潤が臨界点に達するまで H₂SO₄ の濃度を検鏡調整する。

*** 結晶はおそらく金属硫酸塩により細胞膜の一部の分解物が塩析してきたものと考えられる。

超音波振動のために油面におこる波紋の中心部に試験管の底をひたし、そのなかに油面の位置まで水をそそぎ30分間おいた。水中にこまかい原繊維断片の懸濁がおこるようになれば、原繊維系はじゅうぶんにほぐれて観察しやすくなっている

方法 6. 原繊維系の銀染色

方法 5 で処理した材料を塩化亜鉛ヨウ素液にひたしじゅうぶんに繊維素のヨウ素反応をおこさせたのち、過剰のヨウ素をとりのぞき、塩化亜鉛溶液をろ紙で吸いとり、次の溶液をこれにくわえた。

銀アムモニア 2% 水溶液 1 容積

濃アムモニア水 28% 1 "

はじめに白色の塩化銀沈殿が多量にできる。つぎに黒紫色が抜けたころ、上液をじゅうぶんに添加すると、塩化銀はアムモニアに溶解しヨウ化銀がのこる。これをじゅうぶんに水洗し、材料が黄色になったら水洗をやめ、ペロナ現像液により銀を還元する。銀の粒子によって紅緑色の二色性がみえてきたら水洗し、定着液に浸す。

す。あとしばらくヒドラジン溶液につけて放置すると原繊維系の微細な表面が水と明瞭な界面をへだてて観察しやすくなる

方法 7. 顕微鏡による観察

以上の処理によって定量的位相差顕微鏡下において多くの構造的要素の観察が可能となる。しかしより微細な構造的要素は、電子顕微鏡によらなければならないことはもちろんである。以上を下に表に要約する。

本文中に引用した原繊維集束の電顕像は繊維素の膠状液から脱水によって得られた試料を、本学の電子顕微鏡で写したものである。なお綿毛原繊維集束の電子顕微鏡的解析の詳細は、べつの機会に報告する。

結 果 と 考 察

ワタの種子の毛は一本が単一の細胞からなるといわれている。その細胞膜は生きていた状態もしくは乾燥した状態では、光学顕微鏡下で膜として

I 原繊維系の精製	(1) ワックス除去	アセトン エーテル
	(2) 完全物質分解	KCl・ZnCl ₂ H ₂ SO ₄
	(3) 分解物質溶解	エタノールアミン ヒドラジン
	分解物質洗滌	アムモニア水 水
II 原繊維系の化学的拡張, 洗滌	(4) 原繊維系の膨潤による拡張	H ₂ SO ₄ 酢 酸
	残存物質溶解	エタノールアミン ヒドラジン
	原繊維系洗滌	アムモニア水 水
III 原繊維系の物理的拡張	(5) 原繊維系の機械的分散による拡張	超音波
IV 原繊維系の銀染色 (界面)	(6) 原繊維系銀染色	
	ヨウ素吸着	塩化亜鉛ヨウ素
	ヨウ化銀付着	濃アムモニア性酸化銀
	銀還元	ペロナ現像液+ヒドラジン
V 観 察	(7) 位相差顕微鏡による観察	水中

均一な構造にみえる。

しかし光学顕微鏡下でも綿毛の外形として、 $1 \sim 1.5$ の倍率をあげることができる。使用した綿毛の長さは乾燥状態で $2 \sim 2.4$ cm., 幅は乾燥状態で $10 \sim 15 \mu$, 水で湿らせた状態で 20μ , 厚さは湿らせた状態で 8μ ぐらいで、リボン状を呈する。しかし詳細に観察すると 1 コの細胞の幅が狭いところになって、または異なった細胞のそれぞれによって一定の周期をもった節があり、節間の長さは $200 \mu \sim 1200 \mu$ ぐらい (Fig. 1. A, B, C) のものがある。

細胞膜のうすいものでは、節間部が扁平な紡錘状をして、それらが同一平面上にならんでいるか (Fig. 1. A) または隣接する節間部とたかいたに 90° 廻転した面上に配置しているもの (Fig. 1. B) がみられる。

ゆえに綿毛の輪郭が波状をしており、したがって節のふくらみやくぼきは節間部と異なってくる。

これらの形状とは無関係におなじ綿毛にらせん状のうねれが観察できる。このうねれは乾燥状態ではなはだしく、湿度が増すにしたがってねじれはもとよりうねれだけの構造が弱くなって来る。

またある場合には波状が顕著でなく、比較的に一様な幅もしくは太きをもった節間部のなかい細胞がある。これらの細胞は節間のくびれが複雑な構造をもち、節間部とはほぼ同様な太きになっている (Fig. C)。

じゅうぶん成熟した綿毛の表面には、不明瞭ではあるが、長軸に傾斜して走る平行な多数の線条がみられる。ことにスタン III でワックスなどを染めるとその線条の溝のなかに濃く染まる場所がある。綿毛はすでに述べたような方法によって物理化学的な処理を受けると、膜の骨格をなす纖維素原纖維だけからなる樹枝状の原纖維系の構造が明らかになる (Fig. 3., Fig. 4 A)。

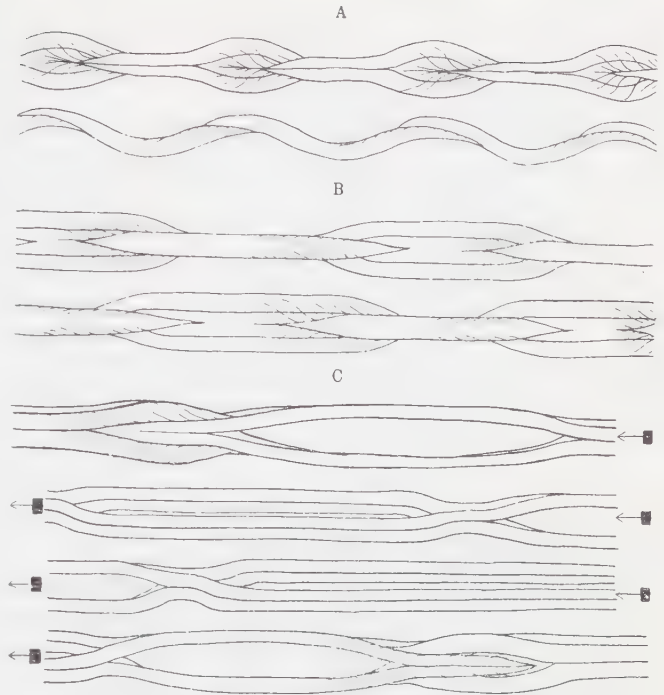


Fig. 1. A. 上は 1 節に 1 対の左右対称対をもつ綿毛の模式図。下は上図の側面図。
B. 上は 1 節に 1 対の背腹対称対をもつ綿毛の模式図。下は上図の側面図。
C. 1 節に 4 本の原纖維集束をもつ綿毛の模式図。

原纖維はほぼ同様な形状の単位原纖維集束 (全長 $200 \mu \sim 1200 \mu$ で節間の長さにはほぼ等しい (Fig. 5)) が多数くみこまれてできている。単位原纖維集束は両端部がほとんど上下対称に掌状 (羽毛状) を呈したこまかい原纖維の分枝で、中央部は 1 本の太い (幅 8μ) 腕状の原纖維の束である。

おのおのの節間部では、2 本 (1 対) または 1 本 (2 対) の単位原纖維集束が長軸に平行にならんでいる。2 本の場合にはたかいたに左右対称に平行にならんでいる時と背腹対称にむきあっている時とある (Fig. 1. A, B)。4 本の場合には左右対称対がたかいたに背腹対称に位置する (Fig. 1. C)。左右対称対の腕状部は平行に接しているので $4 \mu \times 2 = 8 \mu$ の幅をもち、背腹対称対では細胞腔をはさんで対立的に位置し、腕と腕との間には腕状部からわずかに分枝する原纖維によって薄い膜壁部を形成している。

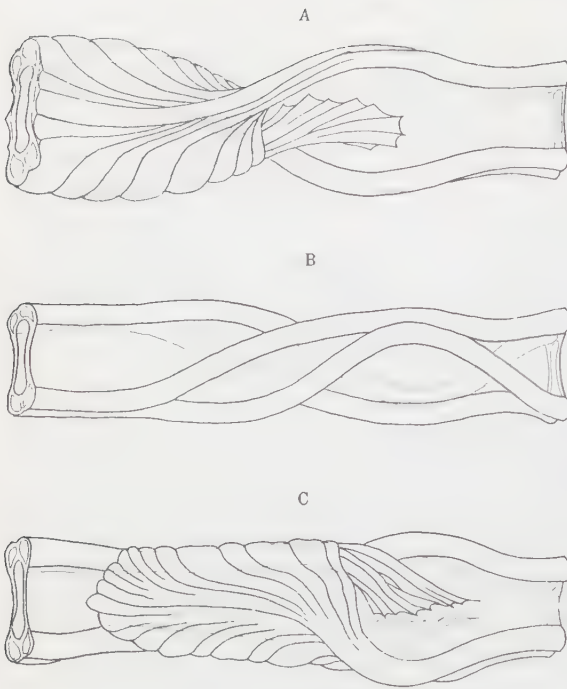


Fig. 2. A. 1節に 4 本の原繊維集束をもつ綿毛の模式図。上端の掌状部。
B. 同上 中間の腕状部。
C. 同上 下端の掌状部。

1 対のみの背腹対称対の掌状部は節のところでたかいた合掌形をなし (Fig. 1. B), 1 対のみの左右対称対は、対の原繊維集束間では合掌形をとらないで隣接節間の対の掌状部と 180° 廻転した面で逆位の合掌形をとる (Fig. 1. A)。1 節に 2 対の原繊維集束をもつものでは、2 対の左右対称型の並立掌状部がそれぞれ背腹対称型に配置し、節部において合掌形をなし、上部隣接節間の下端の合掌部を掌状部と掌状部との間に 90° 廻転した位置で内部にはきんでいる (Fig. 2 A, C)。

上端の掌状部は鱗片状をなして外表に現われている。原繊維の分枝は腕状から掌状に変わる節部で最もいちばん多く、分枝法は叉状分枝および仮軸分枝である。腕 (幅 4μ) は幅 $1\sim 2\mu$ ぐらいの原繊維を叉状に分枝し数回分枝をくりかえすから、結局 $100\text{Å}\sim 250\text{Å}$ ぐらいの原繊維が 2 本ずつ平行に走ることになる (Fig. 4. B. Fig. 5)。垂直裁断線の方に分枝した原繊維はたがいに羽毛

状の原繊維層を形成し、求心方向に分枝した直後の原繊維は中軸に平行な方向に走向を転じ、等深度の原繊維とともに羽毛状の原繊維層を形成するが、遠心方向に分枝した原繊維はさらに仮軸的に分枝をおこない、いったん表面にでてから直後に中軸に対してある角度をもって表面をらせん状に走り、片羽状の原繊維層を形成し、一見外表には連続的ならせん状の原繊維の走向を思わせる構造を形成し、一段内側の層とともに網状を呈する。表面のらせん状の走向は両端掌状部付近で鋭角をしめし、腕の中央にちかづくにつれて垂直となる。上述の分枝構造は腕中央を中点として上部と下部の分枝角度が 180° 異なる。ゆえに表面ちかくの原繊維集束の形状および相対関係はらせん状構造が主になり、相似形は考えられるが、対称形は考えられない。しかし第 2 次肥大生長の進んだ部分では各原繊維集束の形および相対関係はほとんどが中軸に平行な走向をしめし、対称形が主になり、らせん構造はなくなる。

上部隣接節間の原繊維集束の掌状部は、たがいに接する節間において両者の掌状部の分枝の先端がたがいに 180° の方向から交錯するが、間接な連結であって原繊維間には連続関係はない。すなわち原繊維集束はおのおの単位として独立しており、上下の原繊維集束の連絡には軸方向の張力に対し許容性をもたず、むしろ抵抗性をもった力が考えられる。

節間の長いものでは節間の途中で 2 回ぐらい腕の対の組かえがみられることがある (Fig. 2. B)。すなわち左右対称対のわのおのが分かれてむかい側に位置する背腹対称対の対称的な位置にあるものとあらたに左右対称対を形成する。ゆえに節間の距離よりも短い紡錘形の扁平な部分が 90° ずつ廻転した位置にならび、節に類似した形状のものが数個節間部にあらわれる。一見元来の節とみあやまる形状をしめすが、これは腕の対の組かえである。以上の構造には細胞の膨圧に対し抵抗性をもった力が働くと考えられる。

第 1 次細胞膜と第 2 次細胞膜との原繊維系の関係について見解を求めておきたいと思う。綿種皮

にある厚膜柵状組織では、これらの細胞は比較的長い細胞である。これの原繊維集束の配置は、綿毛の場合よりも1節を構成する原繊維集束の数がはるかに多く、それらがたがいに複並立の構成をしめし、しかもただ一つの節間部を有するのみである。原繊維集束の両端は掌状部の原繊維層がたがいに隣接するものと相対して、細胞のカラー (Collar) を形成している。この第2次肥大生長膜の外側に薄い第1次細胞膜があってその原繊維走向は垂直裁断線の方である。第2次肥大生長膜の原繊維系はほとんどすべてが長軸方向に走り、遠心方向に分枝した原繊維の先端は第1次細胞膜の原繊維層の連絡を保っているようであるが、そのくわしい連続関係はまた不明である。

Bailey et al. (1957) はアベナ子葉鞘の表皮細胞で原繊維走向をしらべ、第2次肥大生長膜の原繊維層から分枝した原繊維の先端は第1次細胞膜の原繊維層のなかへ接着するように考えている。

Berkley (1948) は綿毛の節付近では多数の層になることを示し、外側の層から内部の層にゆくにしたがって原繊維の走向が垂直方向から軸方向に順次に変化するという見解をのべている。

Mühlethaler (1949) は第2次肥大生長膜のすべての層が同一の走向をもつことを示し、その走向をしめすことを電子顕微鏡によってみている。

Berkley のあたえた模式図は筆者の観察した綿毛の節付近の構造に相当し、Mühlethaler の見解は節間部の腕のあるところに相当するものと筆者は考える。原繊維集束の電子顕微鏡像 (Fig. 4, B, Fig. 5) による構成要素の測定は、筆者が当教室でおこなった仕事の一つであって、それぞれの数値を概算し次にあげる。

原繊維集束全長 $100\mu \sim 200\mu$

原繊維集束腕幅 $3.4\mu \sim 4\mu$

原繊維の幅 $100\text{\AA} \sim 250\text{\AA}$

ここにあげた原繊維集束は、前にのべた光学的顕微鏡下に観察した単位原繊維集束と相同のものの像であると思われる。ここにのべている原繊維は Mühle-

thaler (1949), Frey-Wyssling (1950) の場合と一致するものといふ一致するものはある。

綿毛細胞膜についての微細構造に因して光学顕微鏡による研究の結果から原繊維集束の形態的概念がほぼあきらかとなった。

以上の研究は東大教授和田文吾博士および田中信徳博士の御指導のもとに東大理学部においてなされた。電子顕微鏡の使用については同大学電子顕微鏡室において佐藤正一氏および坂田茂雄氏の絶大な協力と指導を得たものである。ここに深く感謝を述べたい。

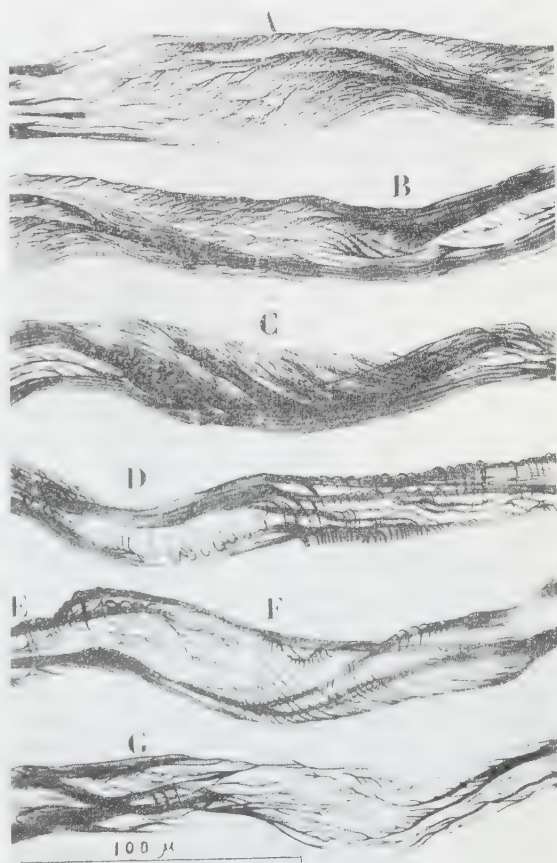


Fig. 3. Fig. 4A の原板より半模式的に描いたもの。

A: 上端掌状部 B: 分枝開始部 C: 腕の組換え D: 中央腕状部 E: 腕の組換え F: 分枝開始部 G: 下端掌状部

Summary

1. The cell walls of the full matured cotton seed hairs were chemically purified and dispersed by the ultrasonics. The structure of the cellulose fibrillar system of the cell walls treated with the above procedure, has been observed under the light microscope and electron microscope.

2. The cellulose fibrillar system is formed by the unit of cellulose fibrillar bundle. One cotton fiber has many segments constructed by 2 or 4 collaterally situated cellulose fibrillar bundles having axially parallel orientation.

3. Upper and lower ends of the bundles in each segment represent plumous ramification (Fig. 4 A, B, Fig. 5) which can be easily distinguished from a rope like middle portion (Figs. 2A, B and C).

4. The cellulose fibril has a diameter of 100 \AA — 250 \AA . The fibrillar bundle has a diameter of ca. 4μ . Full length of the bundle is $100\mu\sim 1200\mu$.

5. One pair of the fibrillar bundles in each segment situated in lateral symmetry or dorsiventral symmetry, but in the case of 2 pairs, the 2 lateral pairs are situated in dorsiventral symmetry (Figs. 1A, B and C).

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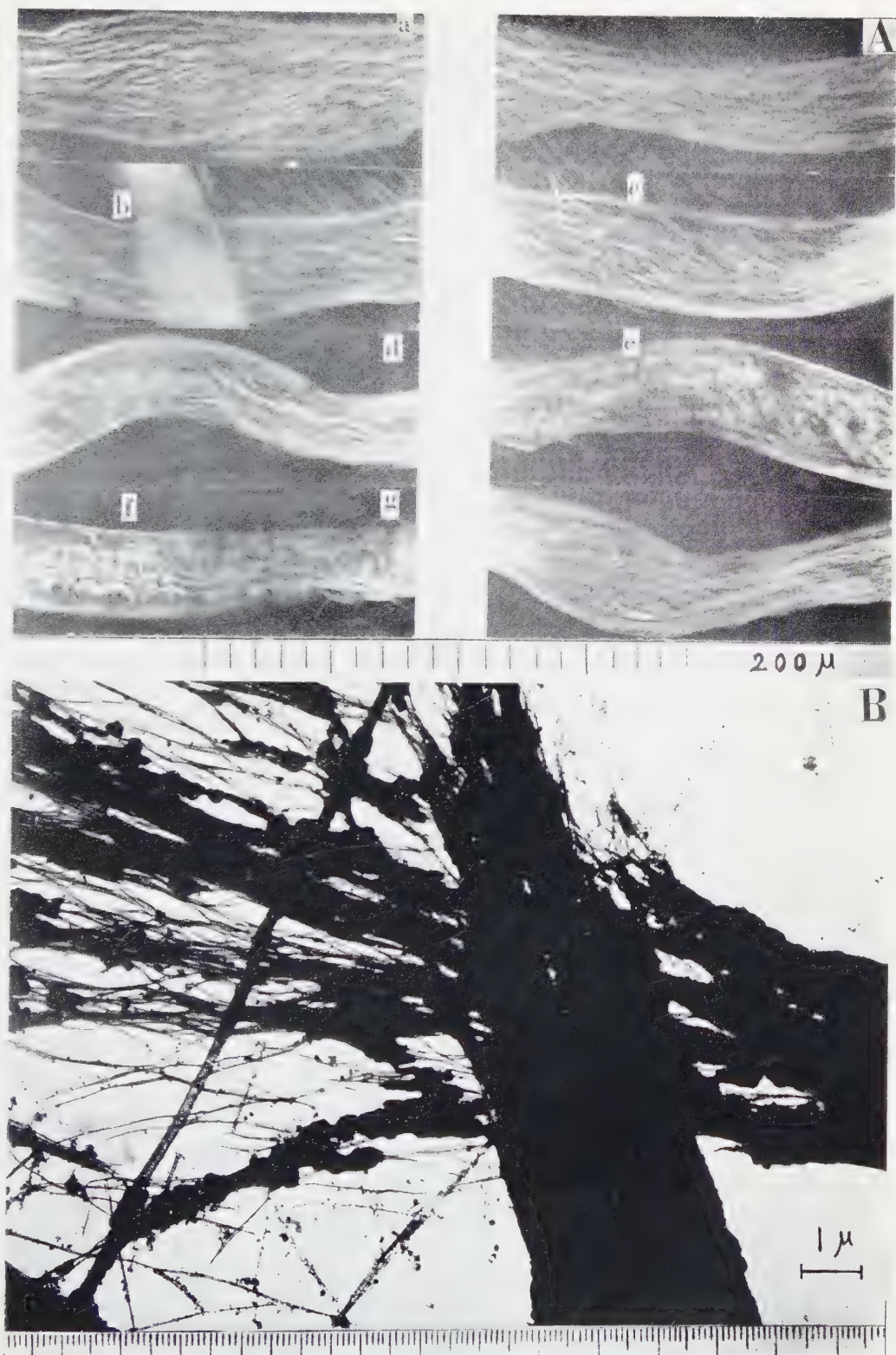


Fig. 4. A: 原繊維系の位相差顕微鏡写真像 a~g は Fig. 3. の A~G に相当する。

B: 原繊維集束およびその分枝をしめす。電子顕微鏡像 ×9000

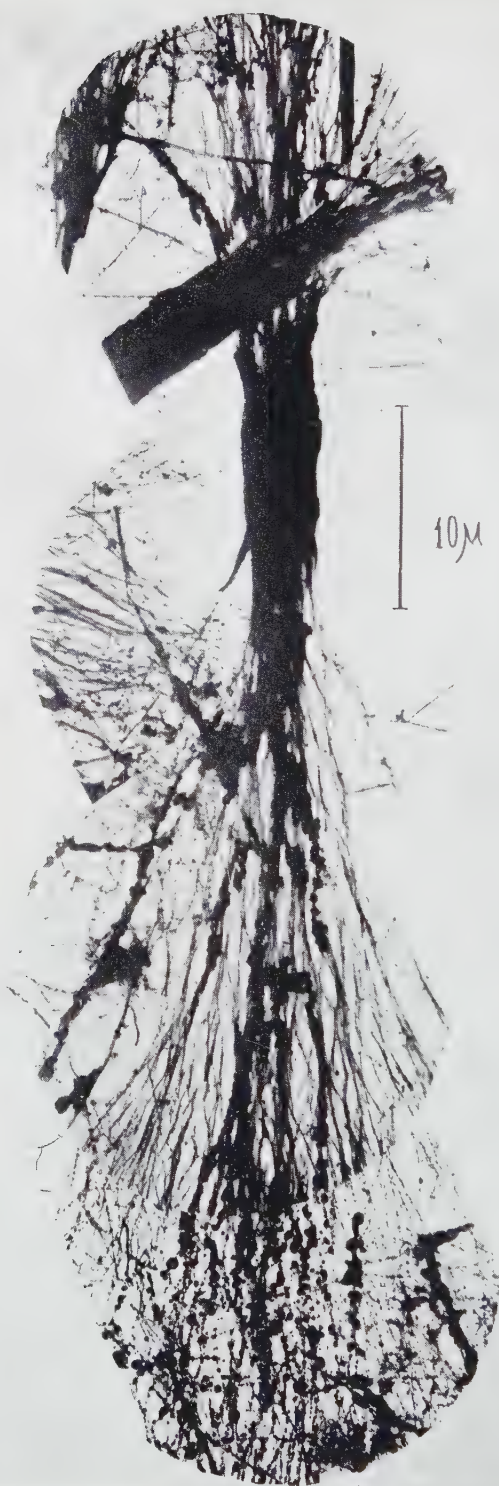


Fig. 5. 単位原纖維集束の電子顕微鏡像 $\times 3000$

雑 録

“インド植物生理学会”の設立

最近小生あてに知人 Delhi 大学の J. J. Chinoy 教授のもとから下記の知らせが送られて来た。

昨 1957 年 8 月 Delhi 大学で「インド植物生理学の最近の進歩」に関するセミナー(会長、ハーヴァード大学 K. V. Thimann 教授)には全インドの各地から植物生理学者が参集したのであるが、そのときそれらの人びとによってこの国における植物生理学の発展を促進するために“インド植物生理学会”を設立する事を決意したのである。そこで本年 1 月 24 日 Allahbad 大学で学会創設のための大会が開催され、会則の承認とともに本年度の役員組織が決定された。

会長; P. Parija 教授 (Utkal 大学) —— 以下、副会長、幹事、理事等の役員には Shri Boshi Sen (Vivekanda 研究所), Shri Ranjan (Allahbad 大学), J. C. Sengupta (カルカッタ, インド植物調査所), J. J. Chinoy (Delhi 大学), K. K. Nanda (Delhi 大学), S. C. Chakravarti (Bhopal), R. S. Chawdhury, (Banaras), B. K. Kar (Barrackpore) T. S. Sadasivan (Madras), P. K. Sen (Calcutta), P. V. V. Seshagiri (Waltair), S. M. Sircar (Calcutta), R. N. Tandon (Allahbad) の諸氏が就任し、また Kenneth V. Thimann 教授 (Harvard 大学), Kurt Mothes 教授 (Halle 大学), F. G. Gre-

gory 教授 (London 理工科大学) らが名誉会員に推戴された。学会発刊のインド植物生理学雑誌 “The Indian Journal of Plant Physiology” の編集幹事は R. D. Asana (New Delhi, インド農学研究所), 編集委員は R. S. Chawdhury (Banaras), I. M. Rao (Agra), R. N. Singh (Banaras), S. M. Sircar (Calcutta) の諸氏である。

Chinoy 氏の同封の書簡によればかの地の人びとは日本インド両国の科学ならびに技術の一層密接な交流に期待し、“インド植物生理学雑誌”も日本の植物生理学者の寄稿を歓迎するといことである。それと同時に Chinoy 氏はインドの植物生理学論文の紹介、抄録、あるいはオリジナル論文等が日本のしかるべき学術誌に掲載されるみちはないであろうかと尋ねて来ている。

「学術的交流はもとより科学者万人のこいわがうところ、またインドにおける植物生理学会の創立はこの学の促進のための一つの機構として同じ学問分野にたずさわっておられる方がたには関心深いものがあると考え、はるかに“インド植物生理学会”の誕生を祝いつゝ筆をとったしだいである。東京大学応用微生物学研究所 田宮 博

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お し ら せ

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On the Seasonal Change of Photosynthetic Activity of Some Deciduous and Evergreen Broadleaf Trees

by Toshiro SAEKI* and Nobuo NOMOTO**

佐伯, 敏昭・野本達夫**：一年生・樹種の光合成活性の季節的変化

Received April 21, 1958

For the causal understanding of the geographical distribution of plant species, the variation of photosynthetic activity in each species must be followed throughout its whole growing period. We have some informations concerned in herbs and grasses, while in the case of trees only a few reports have been presented. For example Willstätter and Stoll worked with *Acer* and *Tilia*, but with the view of clarifying the mechanism of photosynthesis they always measured it under abnormally high CO₂ concentration. Reports by other investigators were almost concerned in restricted period as in summer or winter. Among them Kusumoto's (1957, 1958) are worthy to be noticed.

In the present paper we describe the changing features of photosynthetic activity in deciduous as well as evergreen broadleaf trees under normal air conditions against Willstätter and Stoll's experiments, and discuss the meaning of difference in activity-curves between deciduous and evergreen trees on their lives and geographical distributions, comparing with the Kusumoto's results obtained in a southern part of Japan.

Method

Trees used for measurements were *Zelkova serrata* (deciduous), and *Shiia Sieboldi*, *Pittosporum Tobira* and *Cinnamomum Camphora* (evergreen), growing in the campus of the University of Tokyo, ages of which were all more than 30 years. After cutting off a twig from the southern sunny side of a crown, a test leaf was again cut in water at the base of its petiole for assurance of water absorption, and it was laid in an assimilation chamber moistened with wet filter paper, in a laboratory. The leaf was preilluminated with 15-20 kilolux light for about 30 minutes, being exposed to air-flow from the open air. These treatments were useful to make the stomata widely open in the most species so far tested. For the light source 300 W flood or 500 W spot reflector lamps, sometimes for weak illumination ordinary tungsten bulbs, were employed, combined with sky light. Air temperature in the assimilation chamber

* Botanical Institute, Faculty of Science, University of Tokyo. 東京大学理学部植物学教室

** Biological Institute, Faculty of Arts & Science, Ibaraki University, Mito, Ibaraki. 茨城大学文理学部生物学教室

was adjusted to 25°, but lowered in winter to 20°, or winter optimum temperature for photosynthesis (c. f. Kusumoto 1957). The assimilation chamber was sustained at 10 cm. depth in a water bath, to intercept the excess heat radiation, although sometimes even under such a condition overheating of leaf was observed at strong light intensity, e.g. in 30 kilolux illumination leaf temperature of *Zelkova* detected with a thermocouple was 5° higher than air in the chamber. The net assimilation obtained at high light intensity, therefore, might be estimated somewhat lower on account of respiration activated with the high leaf temperature. In this paper, however, only the air temperature in the assimilation chamber was noticed because it was too complicated to measure every time the temperature at each test leaf.

Photosynthesis and respiration were measured with Boysen Jensen's method (1932) with some modifications. The obtained values were corrected to 0.03 volume percent CO₂. The photosynthetic ability at light saturation, compensation point, etc., of each leaf were determined after defining the light-photosynthesis curve with a series of measurements. Chlorophyll content of leaves was measured after the procedure of Kasanaga and Monsi (1954).

Seasonal change of photosynthetic activity

A. Deciduous tree. In Fig. 1 we summarized the results obtained in the leaves of *Zelkova serrata*, concerning the maximum net photosynthetic ability at 25°, respiration, compensation point and chlorophyll content. The experiments were carried out in 1953, 1954 and partly in 1955. After sprouting of new shoots in late spring, rapid development of photosynthetic activity of all young leaves follows. Early in June it reaches already the maximum of about 6 mg. CO₂/50 cm²/hr. (Fig. 1). In 1953 and

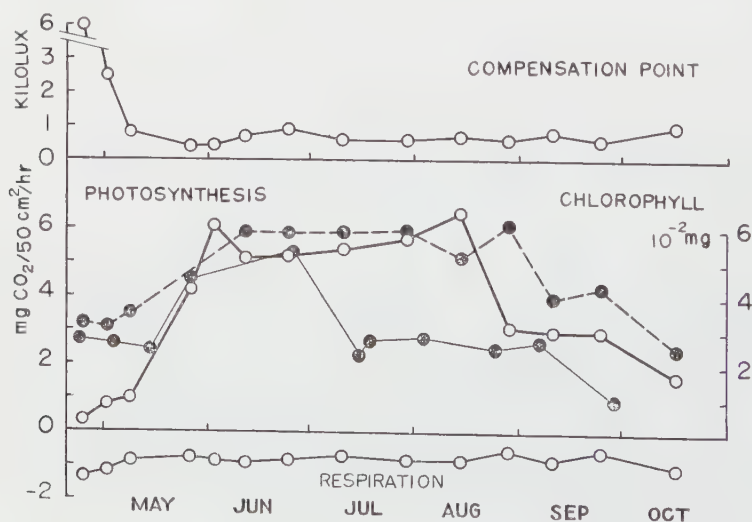


Fig. 1. Seasonal changes of photosynthetic activity, respiration, compensation point and chlorophyll content (broken line) in *Zelkova serrata*. The values were derived from the measurements in 1953, except for the photosynthetic course in 1954 (thin line).

1955, this high value persisted for about two and a half months. Then the activity declined gradually from the middle of August, until all leaves turned yellow losing perfectly their activities and fell in November. In 1954, however, it was somewhat different; so soon did the curve begin to fall as in the middle of July. This might have been caused by the unusually continuous cloudy and rainy days from May to June in that year—in Japan we have ordinarily a rainy season in this time of year. Actually in June 1954 severe leaf fall took place which meant a getting worse of the dry matter economy in the shaded leaves inside the crown, because the daily maximal light intensities measured even in the open were sometimes only 3 kilolux or less.

The trend of the activity curves is principally the same as that [obtained by Willstätter and Stoll (1918) in *Acer* and *Tilia*, except for long duration of high activity for more than 4 $\frac{1}{2}$ months, which seems to be caused by extremely high CO_2 concentration in their experiments (5%). In *Zelkova* the activity above the value of a half of the maximum could extend over 4 $\frac{1}{2}$ months, from the middle of May to the end of September, and it corresponds to the period of mean monthly temperature above 17°.

The dry weight per sq. cm. leaf area increased from 4.2 mg. late in April, to about 8-9 mg. early in June. After about two months only a slight increase reappeared, then the weight decreased with yellowing of the leaves. In the younger age of leaves higher respiration and higher compensation point were characteristic in parallel with lower chlorophyll content and lower photosynthetic activity (Fig. 1). This was more conspicuously observed in a Lammas shoot where leaves are successively arranged from younger to older ones. Light-assimilation curves obtained in four leaves of such a Lammas shoot (Fig. 2 and Table 1) show that with maturing of the leaves the photosynthetic activity and chlorophyll content increased, but the respiration and compensation point decreased. The maximum photosynthetic activity of *Zelkova* leaves changed fairly parallel to chlorophyll content (Fig. 1 and Fig. 3-a). Same relationship in the Lammas shoot appeared itself but gave somewhat indifferent curve (Fig. 3-b). This discrepancy in the two kinds of shoots seems to be rather natural, because Gabrielsen (1948) has already pointed out that the quantity of chlorophyll pigment is responsible not for the maximum photosynthesis but for the linear regression

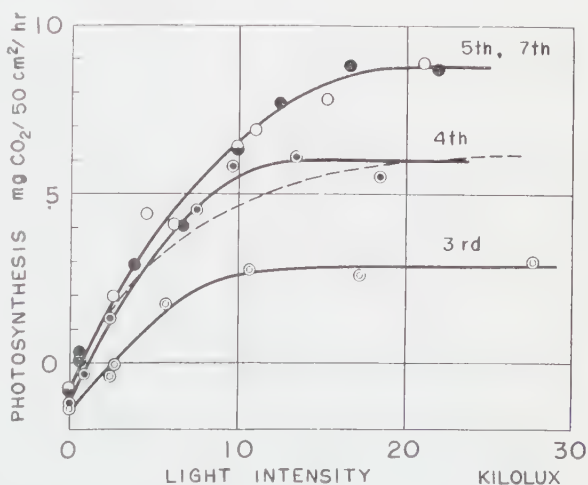


Fig. 2. Light-photosynthesis curves in the leaves of a Lammas shoot. See Table 1. Nodes were numbered from the top to the base. Broken line shows the curve of normal leaves.

Table 1 Leaf size, maximum assimilation, chlorophyll content, respiration and light-compensation point in the leaves of Lammas shoot of *Zelkova serrata*. Nodes were numbered from the top to the base of the shoot.

	Leaf on			
	3rd node	4th node	5th node	7th node
Leaf size (cm ² , unilateral)	8.0	22.8	25.3	27.4
Max. assim. (mg. CO ₂ /50cm ² /hr.)	2.8	6.2	9.0	9.0
Chlorophyll content (γ/cm ²)	7.1	23.0	43.5	60.0
Respiration (mg. CO ₂ /50 cm ² /hr.)	1.3	1.3	0.8	0.7
Compensation point (kilolux)	2.2	1.1	0.6	0.6

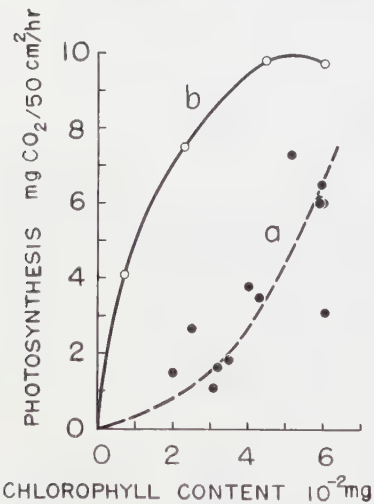


Fig. 3. Relation between chlorophyll content and the maximal gross assimilation. a; annual shoots, b; Lammas shoot.

of photosynthesis curve in weaker illumination. This, combined with higher respiration, causes the difference in the light-photosynthesis curve of very young leaves as well as of yellowish old leaves from that of green shade leaves.

B. Evergreen broadleaf tree. The seasonal change curves of photosynthetic activity of *Shiia Sieboldi*, *Pittosporum Tobira* and *Cinnamomum Camphora* are shown in Fig. 4. In the vegetation period these three species gave similar courses as in *Zelkova*, and the maximum rates were also in the same order, i. e. both the types of trees could assimilate CO₂ almost at the same rate in summer. However, autumnal decline of the activity came somewhat later in the evergreen trees and the activity still remained at a low level throughout winter. In spring the older leaves of *Pittosporum* recovered their activity to some extent, before perishing under newly developing leaves in late spring, while *Shiia* (and also

Cinnamomum) had shown scarcely any indication of recovery in the activity. Low winter temperature near the northern limit of natural distribution of such evergreen trees (Tokyo, 35°40' N, mean temp. in Jan. 3.2°) may be responsible for the depression of the photosynthetic activity, because the low temperature is unfavourable for respiration which most possibly supplies the energy for maintaining the photosynthetic apparatus in active condition. The trees used for experiments were grown near such distribution limit. The photosynthetic activity of *Shiia* in winter was scarcely influenced with varying temperature in wide range from 3° to 25°, except for its slight rise at 20°. The values obtained in December fell in the range of 0.5-1.2 mg. CO₂

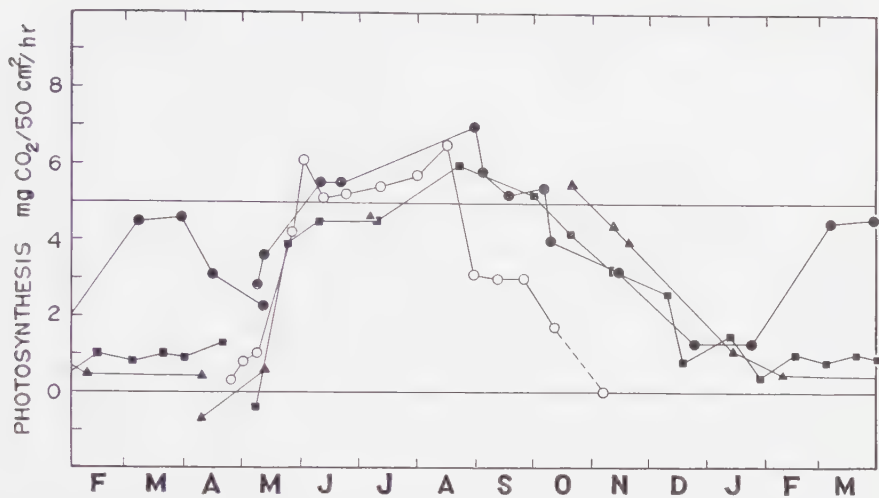


Fig. 4. Seasonal changes of photosynthetic activity in three species of evergreen trees and *Zelkova serrata* (the same as in Fig. 1).

—●— *Pittsoporum Tobira* —■— *Shiia Sieboldi*
—▲— *Cinnamomum Camphora* —○— *Zelkova serrata*

/50 cm²./hr. and were much lower than those of 2-3 mg. measured by Kusumoto (1957) at Kagoshima (31°30' N., mean temp. in Jan. 6.4°) in southern Kyushu, the distribution centre of the evergreen broadleaf forest.

Ecological meaning of the seasonal change in photosynthetic activity

The amount of organic matter produced by leaves is mainly decided by three factors, that is, photosynthetic activity of leaves, duration of high activity and total area of leaves. Supposing the maximum photosynthetic activity as 6 mg. CO₂/50 cm²./hr. in ordinary leaves of deciduous as well as evergreen trees and 9 mg. in Lammas shoot from the results obtained and photosynthesis for 10 hours and nocturnal respiration for 14 hours per day, so the daily dry matter production with open stomata on sunny day in summer will approximately be calculated as b in Table 2. Dry

Table 2. Comparison between dry matter per unit leaf area and produced dry matter per day per unit leaf area.

	Deciduous tree		Evergreen tree
	Annual shoot	Lammas shoot	Annual shoot
a. g. dry matter/m ² . of leaf	90	90	180
b. g. dry matter produced/m ² ./day	6	10	6
c. Ratio between a and b	15	9	30

matter weights per unit leaf area in the Table were based on the data on *Zelkova* as deciduous type and *Shiia* and *Pittosporum* as evergreen one. Some part of the produced matter must compensate for the invested capital or the dry matter necessary for replacement of fallen leaves. From the Table it may be roughly estimated that the photosynthetic days for the compensation of annual leaves are 15 days in the deciduous type and 30 days in the evergreen, hence, the evergreen leaves must work for maintenance of their leaves at their highest efficiency at least for 15 days more than the deciduous do. Where the winter temperature is enough high to allow effective photosynthesis, it may not be difficult to realize this. The difference in the production and economy between these two types should play an important role in competition between them, and consequently in the distribution of plant species. In this respect it should be remembered that high winter activities remain in *Shiia Sieboldi* and *Cinnamomum Camphora* at Kagoshima, while the summer activities are almost the same as our results (Kusumoto 1957). The leaves of *Cinnamomum* are not much thicker than those of *Zelkova*, but concerning dry matter economy, this may be offset by much low winter photosynthetic activity (Fig. 4 and Kusumoto 1957). In *Lammas* shoots the photosynthetic days for compensation of dry matter are shortened by their high activity up to 9 days, but the duration with this is limited to only a half of that in the normal leaves.

In a plant community not all the leaves can receive full sunlight on account of mutual shading. The total area of openings in canopy through which the sunlight penetrates up to the ground, gives a good measure not only of the light intensity on the forest floor but also of leaf amount. Sky photographs have been taken in *Zelkova* about every two weeks. Early in July the area of the openings in canopy reached a minimum of ca. 13%, and this corresponds to 2.9 in leaf area index (total area of leaves per unit area of land surface), assuming the leaf inclination as 45° . The openings continued in minimum until the middle of September, followed by gradual increase with autumnal leaf falling.

The shade leaf of *Zelkova* showed the maximum photosynthetic activity of 3-5 mg. $\text{CO}_2/50 \text{ cm}^2/\text{hr.}$, and the respiration of 0.3 mg. $\text{CO}_2/50 \text{ cm}^2/\text{hr.}$ (June 1955). As the dry weight of the shade leaves was 3-5 mg. per sq. cm. in contrast to 8-9 mg. of the sun leaves, it is expected that on dry weight basis the photosynthesis is in the shade leaves higher than in the leaves even at strong light intensity. Similar results have been confirmed by Tranquillini (1955) in *Pinus Cembra*.

Summary

Seasonal changes of photosynthetic activity of leaves in *Zelkova serrata* (a deciduous tree) and some evergreen trees, were investigated and meaning of concerning dry matter production was discussed.

1. The photosynthetic activity of *Zelkova* increased rapidly up to its maximum value of 5-6 mg. $\text{CO}_2/50 \text{ cm}^2/\text{hr.}$ later in April and the value persisted for about $2 \frac{1}{2}$

months; afterwards the activity decreased slowly until the defoliation in November. The activity maintained itself above a half value of the maximum for about $4\frac{1}{2}$ months of which mean monthly temperatures were above 17° . The photosynthetic activity changed fairly parallel, but only apparently, to the chlorophyll content.

2. In late spring and summer, the courses of change in the photosynthetic activities in evergreen trees, *Shiia Sieboldi*, *Pittosporum Tobira* and *Cinnamomum Camphora*, were more or less similar as that of *Zelkova*. In late autumn the activity fell to a low level, and it continued throughout the winter, and only in *Pittosporum* the activity recovered before the leaf fall in next late spring.

3. The younger stage of leaf is characterized with a higher respiration and compensation point, and with a lower chlorophyll content and photosynthetic activity. These were clearly shown in the leaves of a Lammis shoot which have generally higher activity than those of ordinary shoot.

4. The weight of the leaves of *Shiia* and *Pittosporum* is ca. 180 g./m^2 ., while that of *Zelkova* is ca. 90 g./m^2 ., hence, to get back these invested capitals the former species must earn about double amount of dry matter. In southern districts this may be easily realized in warmer winter, and which makes the evergreen tree more advantageous in competition with the deciduous tree in natural distribution.

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Developmental Mechanics of Fucaceous Algae X. Structure of the Mucilage Surrounding *Coccophora* Eggs

by Singo NAKAZAWA*

中沢信吾*: フークス科藻類の発生力学 X. スギモクの卵をとりまく粘質物の構造

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When fertilized *Fucus* eggs are forcibly elongated by sucking them into a capillary, having a diameter smaller than that of the eggs, and then jetting them slowly out of it, their developmental axis, the polarity, is determined parallel with the elongation⁷⁾. Another instance of axis determination by external morphology is known in *Sargassum*⁵⁾. That is, in this alga, the egg becomes elongated to an ovate form, pointed on one side, pressed laterally by each other at the time when it is discharged from the conceptacle, and the primary rhizoids are to be formed at the pointed end. In *Coccophora*, another furoid, the majority of the eggs are spherical, and their polarity is determined by the entrance point of spermatozoid¹⁾. Some eggs, however, are more or less elongated as they are pressed by the surrounding mucilage, and their polarity axis is determined parallel with the elongation⁶⁾. The mechanism of that elongation is analysed in this paper from the structure of mucilage surrounding the egg.



Fig. 1. *Coccophora* egg (e) surrounded by mucilage, observed in Indian ink.

Eggs of *Coccophora Langsdorffii*, the material, were discharged from female receptacles cultured in glass vessels at the Marine Biological Station of Asamushi. For revealing its structure, the intact mucilage was stained with 0.01 per cent Congo red and with 0.001 per cent ruthenium red. Both of these were dissolved in sea water. Otherwise, the mucilage was immersed in 0.1 per cent tannic acid solution for ten hours, rinsed for ten minutes, then transferred to 0.1 per cent ferric chloride solution. By this method, the mucilage adsorbed tannic acid specifically, stained black with the iron, so that its structure was made clear.

The outer margin of the mucilage was revealed by putting eggs into sea water mixing Indian ink. By this means it is known that the egg, about 150 μ in diameter, is surrounded by a large mass of mucilage, about 800 μ in diameter, and a part of it

* Biology Department, Yamagata University, Yamagata, Japan. 山形大学生物学教室

is extended to form a stalk of some 100μ in length (Fig. 1). The mucilage can be stained with ruthenium red. The staining, however, occurs not in the overall mucilage mass but in a limited zone so as its extension is about $300\mu \times 700\mu$. Therefore the whole mucilage is divided into the inner and the outer parts, and merely the former is stained with ruthenium red. When the intact mucilage is immersed in ferric chloride after being treated with tannic acid, it is stained black. The staining appears only in the inner part which is stainable with ruthenium red as aforesaid. Upon this occasion, we can clearly distinguish the inner membrane staining deepest of all the other part of the mucilage. This membrane, i.e. the wall of the perioospheric space, is about 2.5μ in thickness and has a number of small pits piercing perpendicular (Fig. 3). The interval between the pits is about 1μ . The outside of that membrane is filled with a thick mucilaginous layer staining in a lighter tone so that it can be distinguished from the inner membrane as well as from the outer mucilage which is unstainable. That is to say, the mucilage mass is composed of three parts, i.e. 1) the inner membrane stained deep black, 2) the middle layer stained in a lighter tone, and 3) the outer mucilage unstainable by tannic acid-iron method. The middle layer consists of many sub-layers so that a number of striations running parallel with the surface can be perceived (Fig. 3). The middle layer extends towards one direction so as to form the axle of the stalk, in which the layer is also stained in a light black tint and there we can see many striations running longitudinally. On these striations, particularly in the stalk, there are a number of small particles staining black. The striations are much more closely compact in the stalk, so that there the striations look like a bundle of stripes. As a result, dynamically, the perioospheric space is more or less distorted to an elongated form. Herein, if the space is comparatively large enough, the egg is free from the shape of that space so that it takes a spherical form. But if



Fig. 2. Staining mucilaginous stalk with Congo red. A) eggs before immersing in Congo red solution, B) stained stalks, C) the same indicating a uniform relation between the egg axis and the direction of the stalk. Note that the stalk is composed of three separate zones, *a*, *b*, and *c*.

the space is comparatively small, the egg is pressed by the mucilage so that it comes to be elongated in the direction parallel with the longitudinal axis of that space, that is, parallel with the stalk. The egg before fertilization is so soft that its form can be altered even by a slight pressure. This relation is revealed when the mucilage is stained with Congo red.

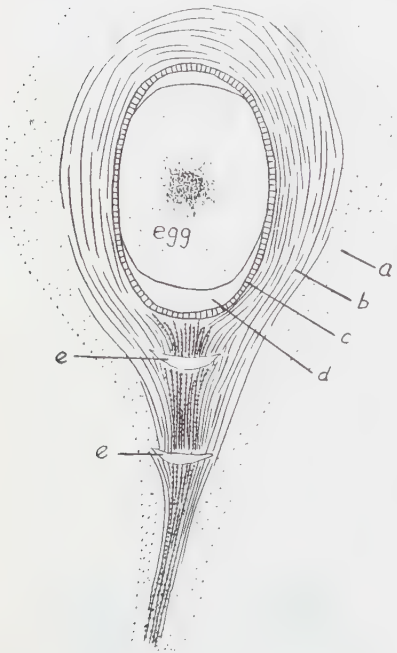


Fig. 3. Schematic illustration of the structure of mucilage. a, outer mucilage; b, middle layer; c, inner membrane; d, perioospheric space; e, gap.

of such eggs is determined in relation to the position of the stalk. Its mechanism was stated above.

When the intact mucilage is kept in 0.1 per cent Congo red dissolved in sea water, the dye is accumulated in the stalk gradually. At first, the stalk axle is stained red, then deep blue-violet particles begin to surround the axle (Fig. 2B, C). The particles are almost of the same spindle-form. What is noteworthy is that the staining appears to be separated into three principal zones, that is, 1) the neck zone which is the smallest, 2) the middle zone, and 3) the tail zone (Fig. 2B). These are separated from each other by unstainable clear gaps, which are also observed in the staining by tannic acid-iron method (Fig. 3). The colour differentiation of Congo red implies that the stalk is composed of the axle, charged negative, and the surrounding mucilage, charged positive electrically. That the egg was connected with the conceptacle by a stalk was reported by many authors^{2), 3), 4)}, and now its structure was made clear by the above staining experiments. In Figure 2 we can see that the axis of the elongated egg is all directed parallel with each stalk. This verifies that the polarity

Summary

1. The mucilage which surrounds the egg of *Coccophora Langsdorfii* is stained with Congo red, with ruthenium red, and with ferric chloride preceded by treatment with tannic acid.

2. The mucilage is composed of the inner membrane, the middle layer, and the outer mucilage. The inner membrane has a number of small pits. The middle layer consists of many sublayers. The outer mucilage cannot be stained with any of the above agents.

3. The axle of the mucilaginous stalk is stained red, and its surrounding zone

deep blue-violet with Congo red. This reveals a positional relation between the egg and the stalk. It is known that the form of the elongated egg is controlled by the pressure, perpendicular to the stalk, of the inner membrane, so that the polarity axis of such eggs is determined parallel with the stalk.

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Promotion of Flower-Initiation by Restraining the Vegetative Growth in the Japanese Radish*

by Hitoshi KOJIMA and Satoshi MAEDA**

小島均・前田敏**：大根の栄養生長抑圧による開花の促進

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Introduction

It is reported that there is an antagonistic relation between vegetative growth and sexual development in plants^{3,4,6,9}. On the other hand, the winter rye "Petkus" initiates flowering at the seventh folial node when the effect of vernalization is optimum, and as the effect of vernalization weakens the number of the nodes up to the first flower increases; whereas even a non-vernalized plant initiates flowering at the 25th node²⁵. It is also known that several plants for whose flower-initiation a low temperature is considered indispensable can be flowered under a suitable condition of other factors, without subjecting them to such a degree of cold that they ordinarily require^{18,19,23,27,29}. These facts lead to the speculation that the retarding of vegetative growth or the aging of the meristem-tissue is favourable for flower-initiation and that cold treatment for vernalization itself can be regarded as a treatment for retardation of growth of the meristem-tissue. From this point of view the authors made several experimentations to examine whether restraining of active cell-division in the meristem-tissue can promote flower-initiation or not.

I. Retardation of growth of growing point by embedding in gypsum

As the material the seedlings of the Japanese radish plant ("Kuroba-minowase", a race of *Raphanus sativus* L. var. *raphanistroides* Makino***) were used. Seeds were soaked in water for 24 hours, then spread on filter paper in a large Petri jar and allowed to germinate. When the cotyledons expanded in a minimum degree possible for the treatment with gypsum (about 1 week after the germination) the

* Contribution from the Botanical Laboratory, Faculty of Agriculture, Kyushu University.

** Botanical Laboratory Faculty of Agriculture, University of Kyushu, Fukuoka, Japan.
九州大学農学部植物学教室

*** It is preferable for this plant to be subjected to a low-temperature in the younger stage of seedling in order to initiate flower, but it can also be flowered by the green plant vernalization. It is said that when it is sowed before the middle of April the bolting is seen, by the effect of the natural low-temperature, but the plant sown after the first decade of May does not practically flower in the Kanto districts (Tokyo and district)⁸.

seedling was so fixed in a bamboo cylinder with solidifying gypsum that the hypocotyl and plumule were compressed by the gypsum but the cotyledons and roots were left free out of the cylinder as seen in the figure. Seedlings thus treated were planted in the garden; apparently the growing points were not allowed freedom of further elongation; the cotyledons grew larger to about twice the breadth and thickness of the normal cotyledon, in compensation for the restrained growth of the growing point. Some 10 days after the plantation the thread which had fastened the two halves of the bamboo cylinder was cut, then in several days the growing point and young leaves expanded so much that the solidified gypsum was broken. The first one or two leaves were slender, wrinkled and thickish; afterwards normal leaves came out. Bolting was examined several times, and bolting percentages were found as follows: Exp. I-IV.

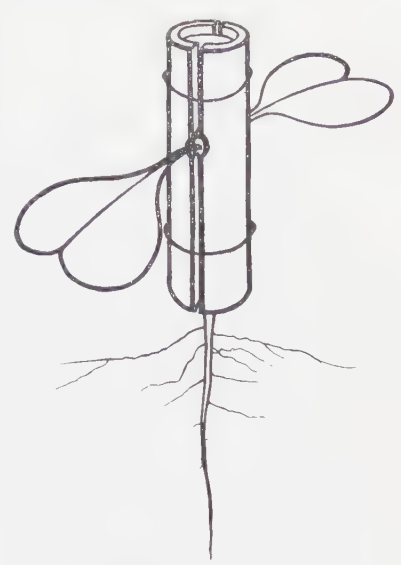


Fig. A seedling fixed with gypsum in a bamboo-cylinder.

Exp. I.

Treatment	Observed:			
	26/V	29/VII	11/VIII	30/IX
Non-treated (control)	* 0.0 (0/60)	0.0 (0/50)	2.2 (1/46)	2.2 (1/46)
Treated with gypsum	0.0 (0/57)	7.2 (4/54)	11.1 (6/54)	18.5 (10/54)

Soaked: 8/V, 1955; Germinated: 9/V: Germinated seedlings placed in room-temperature; Treated with gypsum and planted in the garden: 15/V. * Bolting percentage; [Number of bolted individuals]/[Number of total individuals] is given in parentheses.

Exp. II.

Treatment	Observed:			
	19/VI	2/VII	16/VII	30/VII
Non-treated (control)	0.0 (0/66)	0.0 (0/66)	0.0 (0/66)	3.0 (2/66)
Treated {	hard*	10.8 (5/46)	17.3 (8/46)	26.0 (12/46)
	soft**	7.1 (3/42)	21.4 (9/42)	21.4 (9/42)

Soaked in water: 17/IV, 1956; Germinated: 18/IV; Treated with gypsum, and planted in the garden: 24-26/IV; Thread cut: 6/V. * With hard-kneaded gypsum; ** With soft-kneaded gypsum, which was found also to solidify satisfactorily.

From these experiments it may be said that the gypsum-treatment serves to promote flower-initiation to some extent. The controls (not treated with gypsum) also bolted though rarely; and this may

be explained as follows: the natural low temperature*** may occasionally cover the plants cultivated out of doors, and it may happen that the cold can vernalize some of them in such a degree that they can barely bolt. The percentages of bolting were far smaller in the materials not treated than in the treated ones.

In Exp. IV all the materials failed to bolt, except the 2 plants treated with hard gypsum; this means that the unfavourable high temperature of the summer inhibited vernalization or some physiological changes which must occur in the meristem in order to cause flower initiation. The gypsum treatment was carefully performed but some of the materials may not have been pressed on their growing points exactly enough to answer the purpose; for it was difficult to determine externally whether the technics succeeded completely or not. So if successfully gypsum-treated materials alone could be counted the percentage of bolting might be higher than the figures given in these tables.

II. Inhibition of growth of growing point with maleic hydrazide solution

Seedlings of the Japanese radish were used as materials; they were planted in the garden immediately after germination and about the time when the plumules have slightly appeared (about 5 mm. long) a drop of the solution of maleic hydrazide (OMH 30, prepared by the Otsuka Kagakuyakuhin Kabushikika'sha; abbreviated below to MH) of several different concentrations was applied to the growing point with a small sharp-pointed brush. As a check a drop of water was likewise put on the growing point of other seedlings.

*** The plants passed through several days of minimum temperature below 8° in the field in the course of experimentation, and in fact, 2, 6, 2 and 2 such days respectively in Exp. I, II, III and IV.

Exp. III.			
Treatment	Observed:		
	19/VI	2/VII	3/VIII
Non-treated (control)	0.0 (0/102)	0.0 (0/102)	0.0 (0/102)
Treated {	9 days*	0.0 (0/47)	8.5 (4/47)
	21 days**	0.0 (0/24)	29.1 (7/24)

Soaked in water: 24/IV, 1956; Germinated: 25/IV; Gypsum-treatment: 4/V. * The thread cut 13/V (9 days after gypsum-treatment); ** The thread cut 25/V (21 days after gypsum-treatment).

Exp. IV.		
Treatment	Observed:	
	9/VII	2/VIII
Non-treated (control)	0.0 (0/63)	0.0 (0/63)
Treated {	hard*	0.0 (0/56)
	soft**	0.0 (0/43)

Soaked in water: 7/V, 1956; Germinated: 8/V; Thread cut: 28/V. For * and ** see Exp. II.

In the seedling whose growing point was treated with 1:500 solution of MH the cotyledons became enormously corpulent but no leaf sprouted; and at last the seed-

Exp. V.

Treatment	Observed:		
	25/V	16/VII	10/IX
Water was applied (control)	0.0 (0/26)	0.0 (0/24)	3.8 (1/24)
MH (1:1500) was applied	0.0 (0/12)	41.6 (5/12)	54.5 (6/11)
MH (1:3000) was applied	0.0 (0/16)	12.5 (2/16)	33.3 (5/15)

Soaked in water: 6/V, 1955; Germinated, and set out in garden: 9/V;

Application: two or three times, when the plumule became 5 mm. long.

Exp. VI.

	Treatment	Observed:			
		17/VI	2/VII	11/VII	5/VIII
A	Water was applied (control)	0.0 (0/57)	0.0 (0/57)	0.0 (0/57)	1.7 (1/57)
	MH (1:1000) was applied once (1/V)	0.0 (0/63)	3.1 (2/63)	9.5 (6/63)	14.2 (9/63)
	" " " " " twice (29/IV, 1/V)	4.3 (3/69)	17.4 (12/69)	31.8 (22/69)	39.1 (27/69)
B	MH (1:3000) was applied twice (29/IV, 1/V)	0.0 (0/78)	3.8 (3/78)	10.2 (8/78)	14.1 (11/78)
	" " " " " three times (29/IV, 1/V, 2/V)	0.0 (0/50)	12.0 (6/50)	20.0 (10/50)	30.0 (15/50)

Soaked in water: A on 23/IV and B on 22/IV, 1956; Planted out in garden: A on 24/IV and B on 23/IV.

Exp. VII.

Treatment	Observed:		
	28/VI	11/VII	26/VII
Water was applied (control)	0.0 (0/112)	0.0 (0/112)	2.6 (3/112)
MH (1:3000) was applied once (6/V) and MH (1:1000) was applied twice (7/V, 9/V)	1.1 (1/87)	11.4 (10/87)	20.7 (18/87)
MH (1:1000) was applied twice (6/V, 9/V)	2.6 (2/75)	6.6 (5/75)	16.0 (12/75)
MH (1:3000) was applied once (8/V) and MH (1:1000) was applied once (9/V)	7.8 (7/89)	10.1 (9/89)	13.5 (12/89)
MH (1:3000) was applied once (8/V)	0.0 (0/59)	1.6 (1/59)	6.7 (4/59)
MH (1:1000) was applied three times (9/V, 10/V, 12/V)	1.7 (1/56)	5.3 (3/56)	14.3 (8/56)
MH (1:1000) was applied twice (9/V, 12/V)	2.7 (2/73)	4.1 (3/73)	8.2 (6/73)
MH (1:1000) was applied once (9/V)	2.6 (1/38)	2.6 (1/38)	10.5 (4/38)

Soaked in water: 29/IV, 1956; Planted in garden: 1/V.

ling died. Solutions of MH of 1:1500 and 1:3000 also did not allow the extension of the growing point; and in compensation for that the cotyledon grew big; in about 2-4 weeks a crumpled leaf appeared and then normal leaves expanded. Exp. V-VII were carried out in that way.

Generally speaking, the application of MH solution hastened flower-initiation; the solution of MH of higher concentration within a certain limit was more effective than that of lower concentration, and the more frequently applied the better.

As the seedlings for the above-mentioned experiments were set out in the field relatively late in spring, the effect of the natural low-temperature was perhaps insufficient for vernalization*. Nevertheless, the experiment with materials which had apparently been already vernalized (see Exp. VIII)** also showed some, not very wide, difference in bolting percentage between MH-treated materials and water-treated ones, and there was difference in the number of leaves up to the floral node, which was smaller in MH-treated plants.

Exp. VIII.

Sowed on:	Treatment	Bolting percentage observed on:				Number of leaves up to the floral node (average)
		9/VI	28/VI	5/VII	25/VII	
26/III, 1956	Water applied	19.1 (9/47)	85.1(40/47)	89.3(42/47)	—	29.0
	MH (1:3000) applied twice on and after 9/IV	41.4(17/41)	85.3(35/41)	95.1(39/41)	—	24.2
28/III	Water applied	15.7 (6/38)	57.8(22/38)	76.3(29/38)	—	29.7
	MH (1:1500) applied three times on and after 9/IV	55.0(11/20)	90.0(18/20)	95.0(19/20)	—	22.4
3/IV	Water applied	—	11.4 (4/35)	34.2(12/35)	57.1(20/35)	34.0
	MH (1:1500) applied three times on and after 12/IV	—	46.6 (7/15)	66.6(10/15)	93.3(14/15)	27.4

III. Inhibition of vegetative growth with hypertonic sugar solution

Germinating seeds of the Japanese radish were placed in a dish and sugar solution of several different concentrations was poured into it so that the lower half of each seed was submerged in the solution. To prevent decay, the solution in the dish was renewed every day. The higher the concentration of the solution, the more effectively retarded the growth of the seedlings; the 20% sugar solution scarcely allowed the seedlings to grow for about 7 days. After the finish of the treatment

* The number of cold days of minimum temperature below 8° the materials met with was only 3 in Exp. V and VII and 6 in Exp. VI.

** The number of cold days which the material experienced in the field was 20 in Exp. VIII.

(about 6 or 7 days long) the seedlings were planted in the garden. The bolting percentage of the materials treated with sugar solution was greater than that of the controls steeped in water. The result is given in Exp. IX.

Exp. IX.

	Duration of treatment (in room temperature)	Solution	Observed :		
			27/VI	16/VII	5/VIII
A	19/IV~26/IV	Water	0.0 (0/107)	0.0 (0/107)	3.7 (4/107)
		20% sugar solution	0.0 (0/43)	18.6 (8/43)	30.3(13/43)
B	23/IV~29/IV	Water	0.0 (0/61)	0.0 (0/61)	4.9 (3/61)
		10% sugar solution	0.0 (0/83)	8.4 (7/83)	21.6(18/83)
		15% " "	0.0 (0/47)	6.3 (3/47)	25.5(12/47)
		20% " "	0.0 (0/44)	2.2 (1/44)	18.1 (8/44)

Germinated: A, 17/IV, 1956; B, 21/IV, 1956. The materials met with 6 (in A) or 5 (in B) cold days of minimum temperature below 8° in the field.

Discussion

Treatment with gypsum gives pressure on the tissue of the growing point and restrains the elongation of that part and, on the other hand, by arresting the cell-division in the meristem-tissue¹⁴⁾ it impels the tissue to maturity.

About the effect of MH on the vegetative organs, seeds, roots, leaves or stems, it is said that MH retards flowering^{7,12,21,22,30)}; the present writers also observed that inhibition of growth of the entire plant caused the retardation of not only vegetative growth but also flower-initiation (see Exp. X)*. In this case the seeds, immediately after germination, were so dipped in the solution or in water of room-temperature that only one half of each seed was submerged, and were left as they were for 2 days; then they were planted in the garden. As it was early in the spring the seedlings must to some extent have been vernalized by the low-temperature** in the open air. Thus, in the two sets of experiments, the water-treated materials bolted at the rate of 95.5% and 33.3% respectively; whereas the MH-treated ones only at

* Exp. X.

	Date of germination	Treatment (soaked in the solution or water, for 2 days)	Bolting percentage surveyed on:			
			9/VI	2/VII	13/VII	26/VII
A	25/III, 1956	Water	20.0 (9/45)	77.7 (35/45)	91.1 (41/45)	95.5 (43/45)
		MH (1:3000)	3.2 (1/31)	35.4 (11/31)	38.7 (12/31)	51.6 (16/31)
B	7/IV, "	Water		25.7 (17/66)	33.3 (22/66)	
		MH (1:3000)		4.7 (2/42)	4.7 (2/42)	

** The materials passed through the days of minimum temperature below 8° on the field 20 times (in A) or 14 times (in B).

51.6% and 4.7%; namely, the MH-treatment retarded flower-initiation. It seems that the plants which had their vegetative growth too heavily inhibited by several unfavourable physiological effects of MH^{10),11),31)}, must secondarily have been delayed in flowering¹³⁾. But application of MH only to the plumule inhibited further elongation of that particular part and made the tissue there old, and the other parts of the seedling, namely, cotyledons, root, etc., were not so retarded. The cotyledons, on the contrary, compensatively enlarged; the plant as a whole was not perceptively delayed in its growth.

The result of the experiment with sugar solution also leads to the same assumption, though in this case the solution must act osmotically¹⁴⁾.

Of course there are many problems to be discussed in the present experimentations. For instance, whether Ca- or SO₄-ions have really no effect upon the materials^{24)*}, what is the antagonistic relation between MH and auxin or auxin-level^{16),20)}, whether sugar acts simply osmotically, not as a nutritious substance^{2),17),26)}, etc. Nevertheless, it can very likely be said that those experimentations contribute something to the speculation that the aging of the growing point or the advance in physiological age of the meristem is favourable for flower-initiation. It is imagined that it may be in those conditions that "vernalin" (if it is really in existence) is formed or, in other words, that the state of protoplasm in the meristem is turned into the pattern essential for the course of flower-initiation²⁸⁾, though not so easily as in the conditions induced by the low-temperature treatment. The flower-initiation of aged cereals without cold-treatment as well as of plants subjected to low-temperature, which, as a necessary consequence, retards the growth of the growing point, may also be regarded as a phenomenon resulting, at least in part, from the same mechanism, namely, advancement of physiological age of the meristem in the growing point.

Summary

1. As the material the Japanese radish was used.
2. Retardation of the growth of the growing point by embedding in gypsum causes flower-initiation to some extent, though the plant is not exposed to cold.
3. Retarding the growth of plumule by application of maleic hydrazide solution to it, causes flower-initiation in some measure, though the plant is not subjected to low temperature.
4. Inhibition of vegetative growth by using hypertonic sugar solution leads to flower-initiation in some degree, in spite of no cold-treatment.

* An experiment was conducted parallel with the Exp. II. In this experiment, instead of pressing the material with hardened gypsum, the lower half of the seedling was dipped in a solution saturated with gypsum for 12 days and then the plant was set out in the garden. The bolting percentage obtained on 13/VII was 0.0 (0/92) and afterwards it became 6.5 (6/92) on 26/VII. The difference of the figures of bolting percentage between the control (not treated with gypsum) in Exp. II and the material of this experiment may perhaps be insignificant.

5. The writers advance a speculation that the aging of the protoplasm in the tissue of the growing point is probably favourable to inducing flower-initiation.

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Effect of Dark Treatment, Given to Different Parts of a Leaf, upon Flowering Responses in *Pharbitis Nil*

Shun-ichiro IMAMURA*, Atsushi TAKIMOTO* and Mitsuro OKUDA**

今村 達一郎*・滝本 敦*・奥田 光郎**：葉一部分の暗処理によるアサガオ開花，ニホヒ：主眼

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In short day plants the dark period must last beyond a definite duration to be effective on floral initiation. The effective duration in a given plant is rather constant under given internal and external conditions. The dark period of less than the critical duration cannot induce flower initiation, even if repeatedly given. It was reported that dark treatment of sufficient duration, given to a portion of a leaf can give rise to flower initiation in some short day plants. According to Cajlachjan, *Xanthium* and *Perilla* initiate flower buds if the basal half of a leaf is subjected to short day, whereas it is not the case when the apical half is treated^{2), 3)}. *Kalanchoe Blossfeldiana* is also reported to form flower buds in response to partial treatment of a leaf⁶⁾.

In the previous papers it was reported that the critical dark period for *Pharbitis Nil* lies between 8 to 9 hours⁷⁾ and that even a leaf surface drastically reduced to 1 cm². can induce photoperiodic responses¹⁰⁾. In the present paper experiments are reported in which the dark periods are given to different part or parts of a single leaf using various methods.

Material and Methods

In the majority of experiments a young plant of *Pharbitis Nil*, strain "Violet"⁷⁾ was deprived of its main axis and all leaves except one were removed. The remaining leaf and its axillary bud served as donor and receptor. The darkening of a definite part of the leaf was done by adjusting a folded black, light-proof paper to both leaf surfaces by means of metal clips. Special attention was paid to adjust the paper to the leaf surface as closely as possible. To give dark treatment to a whole leaf a bag of light-proof paper was used, as in experiments previously reported⁸⁾.

Results

I) Effect of dark treatment of one half of a leaf. Dark treatment was given to the basal or apical half of a leaf from 4 p.m. to 8 a.m., and its effect was compared

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.
京都大学農学部応用植物学研究室

** Biological Institute, Yoshida College, Kyoto University, Kyoto, Japan.
京都大学教養部生物学教室

with that of a dark treatment given to the whole leaf. The results obtained are represented in Table 1. In one experiment in which treatment was given for 5 days, all the treated plants initiated flower primordia, but the number of plants with terminal flower differed markedly in each lot. Ten out of 12 plants, in which the whole leaf was treated, and 4 out of 12 plants in which the basal half of a leaf was treated, initiated terminal flowers. None of 12 plants in which the apical half of a leaf was subjected to darkness, initiated a terminal flower. Another experiment, in which the short day treatment was given for 3 days, gave quite similar results.

II) Effects of two dark treatments of subcritical duration given successively to basal and apical halves of a leaf. In this experiment it was examined whether the effects of two dark periods of shorter than the critical duration given successively to different halves of a leaf can sum up to induce flowering. The apical or basal half of the donor leaf was darkened for 8 hours from 4 p.m. to 0 a.m. and just before the removal of the cover the remaining half was enclosed for the same duration, i. e. from 0 a.m. to 8 a.m. of the next day. In some experiments the dark treatment was started from 8 a.m. so that the plant was maintained in darkness during day time. Special attention was paid that the two darkened areas do not overlap at the border line. After the procedure was repeated 3 or 5 days, the plants were grown under

Table 1. Effect of dark treatment given to a half of a leaf

Start of dark treatment	Duration of dark period × No. of dark treatments	Responses	Portion of the leaf darkened			Light control
			Whole leaf	Basal half	Apical half	
July 17	16 hours × 5	No. of flowering plants / No. of plants observed	12/12	12/12	12/12	0/12
		No. of plants with terminal flower	10	4	0	0
August 23	16 hours × 3	No. of flowering plants / No. of plants observed	16/16	32/32	32/32	0/12
		No. of plants with terminal flower	10	13	0	0

continuous illumination. Four lots of plants served as controls. In the first control lot the whole leaf, in the second the basal half and in the third the apical half of the donor leaf were subjected to darkness for 16 hours. The last lot remained untreated and left on a continuously illuminated bench. The results are represented in Table 2. It is noticeable that the plants used in different experiments significantly varied in their sensitivity to the same photoperiodic treatment. The cause of this variation can not be elucidated without further experimentation, but it seems highly probable that this may be due to the environmental factors prevailing during each experiment.

Table 2. Effect of successive dark treatment of subcritical duration given to the different parts of a leaf. I.

Start of dark treatment	Number of treatment	Responses	Method of dark treatment					Light control
			Basal and then apical half successively, 8 hours' dark period each	Apical and then basal half successively, 8 hours' dark period each	Whole leaf 16 hours' dark period	Basal half 16 hours' dark period	Apical half 16 hours' dark period	
4 p.m. July 7	5	No. of flowering plants No. of plants observed	17/20	16/19	19/19	19/19	20/20	0/19
		No. of flowers	60	61	128	112	122	0
4 p.m. October 8	3	No. of flowering plants No. of plants observed	0/20	0/20	5/20	1/20	3/20	0/19
		No. of flowers	0	0	7	2	4	0
4 p.m. August 22	3	No. of flowering plants No. of plants observed	1/20	3/20	18/19	18/20	17/20	0/20
		No. of flowers	1	3	119	67	68	0
8 a.m. July 11	3	No. of flowering plants No. of plants observed	0/20	0/20	8/20	3/20	2/20	0/20
		No. of flowers	0	0	13	4	3	0

Table 3. Effect of successive dark treatments of subcritical duration given to different parts of a leaf. II.

Start of dark treatment	Duration of dark period × No. of treatments	Responses	Method of dark treatment					
			Basal and then apical half successively, 1/2 of the total duration each	Apical and then basal half successively, 1/2 of the total duration each	Whole leaf total duration	Basal half total duration	Apical half total duration	Light control
5 p.m. Aug. 7	14 hours × 5	No. of flowering plants	11/20	7/20	Control 1	Control 2	Control 3	Control 4
		No. of flowering plants observed						
					18/18	17/19	19/19	0/19
5 p.m. Aug. 17	14 hours × 5	No. of flowers	26	21	138	85	99	0
		No. of flowering plants	2/19	5/19	19/19	18/19	20/20	0/19
		No. of flowering plants observed						
8 a.m. June 21	14 hours × 3	No. of flowers	2	8	135	100	84	0
		No. of flowering plants	0/20	0/20	17/20	11/20	8/18	0/19
		No. of flowering plants observed						
8 a.m. July 15	14 hours × 3	No. of flowers	0	0	42	26	26	0
		No. of flowering plants	0/20	0/20	3/20	1/17	3/20	0/20
		No. of flowering plants observed						
8 a.m. July 22	14 hours × 3	No. of flowers	0	0	8	1	6	0
		No. of flowering plants	0/20	0/20	2/20	3/20	0/20	0/20
		No. of flowering plants observed						
8 a.m. Aug. 22	12 hours × 5	No. of flowers	0	0	6	7	0	0
		No. of flowering plants	0/20	0/20	16/18	0/19	6/19	0/19
		No. of flowering plants observed						
		No. of flowers	0	0	47	0	6	0

Despite the variation in sensitivity, the general tendency in the responses to different experimental treatments is obvious. The plants of which the whole donor leaf was subjected to darkness for 16 hours, initiated the largest number of flower primordia. The darkening of a half leaf for 16 hours is not so effective as that of the whole leaf, especially when given for 3 days. In the plants in which the basal or apical half of the donor leaf was darkened for 8 hours and immediately thereafter the remaining half was darkened for the same duration, i. e. one half of the leaf area received in one day in total 16 hours' darkness, a considerable number of flower primordia were initiated only when the plants used had high sensitivity. From the results we may conclude that the photoperiodic induction caused by dark treatment of subcritical duration in different parts of a leaf can sum up only to some extent. The dark treatment must be given to a definite part of a leaf uninterruptedly in sufficient duration in order to bring about a pronounced photoperiodic effect. The same is the case with all experiments represented in Table 3, where the total duration of darkness was 14 and 12 hours.

III) Effect of two dark treatments of subcritical duration given successively to different leaves. The effect of two dark periods of subcritical duration given successively to two adjacent leaves was also studied. The plant was deprived of all buds and leaves except the first and the second leaves and the axillary bud of the latter. From 5 p.m. to 0 a.m. the second leaf, from 0 a.m. to 7 a.m. the first leaf were enclosed in light-proof bags, while the other leaf was subjected to light. This procedure was repeated for 5 days. In another experimental lot dark treatment was given at first to the first and then to the second leaf. Five lots were used as controls; in two the first leaf and in other two the second leaf alone was subjected to 7 and 14 hours' darkness respectively. The remaining fifth lot served as light control, having received no dark treatment. All plants which received a 14 hours' dark period initiated

Table 4. Effect of successive dark treatments of subcritical duration given successively to different leaves for 5 days. Dark treatment started on 18th of August.

Responses	Method of dark treatment						
	2nd and then 1st leaf successively, 7 hours' dark each	1st and then 2nd leaf successively, 7 hours' dark each	2nd leaf 14 hours' dark period	1st leaf 14 hours' dark period	2nd leaf 7 hours' dark period	1st leaf 7 hours' dark period	Light control
	Control 1	Control 2	Control 3	Control 4	Control 5		
No. of flowering plants No. of observed plants	1/17	0/18	10/10	10/10	0/7	0/8	0/19
No. of plants with terminal flower	0	0	4	2	0	0	0
No. of flowers	2	0	98	66	0	0	0

flower primordia. The treatment of the second leaf was more effective, and brought about more flower buds and terminal flowers. This is in agreement with the results reported by many authors which indicate the significance of the age of the leaf in photoperiodic induction^{1),4),7),13)}. As expected from the foregoing experiments, a summation of induction caused by 7 hours' darkness in two adjacent leaves occurred only to a very slight extent. Only one out of 35 plants produced 2 flower primordia, as shown in Table 4.

General considerations

It is a well accepted view that the photoperiodic induction in short day plants is caused by changes taking place both in the light and in the dark periods. The latter can only be effective when it is prolonged beyond a definite duration. The experiments reported in the present paper show that dark treatment of a portion of a leaf is effective also in *Pharbitis* as in *Perilla*, *Xanthium* and *Kalanchoe*, even if the remaining portion is exposed to light^{2),3),6)}.

It was also proved that the dark treatment must be applied to the same leaf or to the same portion of a leaf uninterruptedly beyond a definite duration in order to cause pronounced flowering response. Two dark periods slightly shorter than the critical one, applied successively to adjacent parts of a leaf, are not so effective as the application of one dark period of double duration to one part of a leaf. This may be due to the fact that the reaction taking place in the dark does not proceed linearly in relation to the duration of darkness but has a pronounced lag phase. At the beginning of the dark period the reaction velocity may be slow, and increase more and more as the dark period proceeds. Thus in the first several hours the reaction brings about only a slight effect but in the hours following the first 7 or 8 hours it may give rise to a pronounced effect.

The same considerations may be adopted to explain the fact why in many short day plants the duration of a dark period sufficient to cause minimal responses and that of the maximum effectiveness are not very different. In Biloxi soy bean the minimal duration of darkness for flower initiation was reported to be 10 hours and that of maximum response 12 hours⁵⁾. This is also the case with *Xanthium*. The dark period must last beyond 9 hours to be effective and the maximum response is obtained by a dark period of 12 hours⁵⁾.

In *Perilla* and *Xanthium* the treatment of the basal half of a leaf is more effective than that of the apical half, indicating that the photoperiodic induction brought about by the exclusion of light may be destroyed in the illuminated mesophyll^{2),3)}. In *Pharbitis Nil*, however, no significant difference can be observed in the effectiveness according to the leaf portion subjected to dark treatment. This may be in accordance with the fact previously reported that in this plant the effect of a dark period of sufficient duration is not destroyed by a following light period⁹⁾.

Summary

1) When two successive dark periods of subcritical duration—for example 8 hours—are applied successively to the apical and then immediately hereafter to the basal half of the same leaf, and this is repeated for several days, no flower primordia or a few only are initiated. The above mentioned effect is very weak in contrast to plants in which the basal or apical half of the leaf is subjected daily to one dark period of two fold duration—for example 16 hours. Two successive inductions caused by dark periods of subcritical duration applied to two different parts of one leaf can initiate a considerable number of flower primordia only when the plant used has high sensitivity.

2) The effect of two dark periods of subcritical duration given successively to two adjacent leaves is not so effective as the application of a dark period of double duration to one leaf.

3) The phenomenon may be explained by the assumption that the reaction taking place in the dark does not proceed linearly in relation to the duration of darkness but has a pronounced lag phase.

4) In contrast to *Perilla* and *Xanthium*, no significant difference could be observed in *Pharbitis Nil*, between the dark treatment of sufficient duration to the apical and to the basal half of a leaf.

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Some Characteristics of Photosynthesis of Fresh Water Phytoplankton

by Shun-ei ICHIMURA* and Yusho ARUGA**

市村俊英*・有賀祐勝**：湖沼の植物フランクtonの光合成の二つの性質

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The photosynthetic activity related with the environmental factors makes the indispensable background for the analysis of primary production of the ecosystem. In the ecological study of organic matter production in lakes, it requires the information on such characteristics of natural phytoplankton. Several excellent studies have been done on these problems, but so far not enough data seem to be collected to elucidate thoroughly many problems still remaining. As noted by many investigators, the photosynthesis of natural phytoplankton differs in seasons and in waters as well. From field survey alone, it is very difficult to make any confirmation of what sort of factor is most essential for photosynthesis as the limiting factor, or how each factor can affect the primary production. Therefore, in order to get quantitative information on the photosynthetic characteristics of natural phytoplankton, it is rather desirable to investigate the photosynthesis under controlled conditions in a laboratory, and the present study was pursued on this line.

1. Methods

Methods used in the present study were similar to the one described previously (Ichimura & Saijō 1958). As for the materials raw waters containing phytoplankton were collected from several lakes in prefectures neighbouring Tokyo. The photosynthesis was measured under laboratory conditions, using O_2 -, and ^{14}C - method. The amount of phytoplankton was estimated by that of chlorophyll.

2. The relation of photosynthesis to light intensity

Lake waters used in this experiment were sampled from the surface of three eutrophic lakes, Kasumigaura, Teganuma and Jōnuma, where dominant phytoplankters growing densely in a gregarious state were *Microcystis* sp., *Fragilaria* sp., and *Chlamydomonas* sp., respectively. Differences in the amount of nutrient materials in each sample were scarcely found. Some of chemical elements were as follows: NH_4-N was 0.1—0.3 mg./l., PO_4-P 0.05—0.08 mg./l., and total carbon 40—50 mg./l. Sample

* Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan. 東京教育大学理学部植物学教室

** Biological Institute, The Osaka University of the Liberal Arts and Education, Tennoji, Osaka, Japan. 大阪学芸大学生物学教室

waters filled in 100 ml. clear glass bottles were illuminated at various light intensities with a 500 W flood lamp in a water bath at 20° for 3 hours.

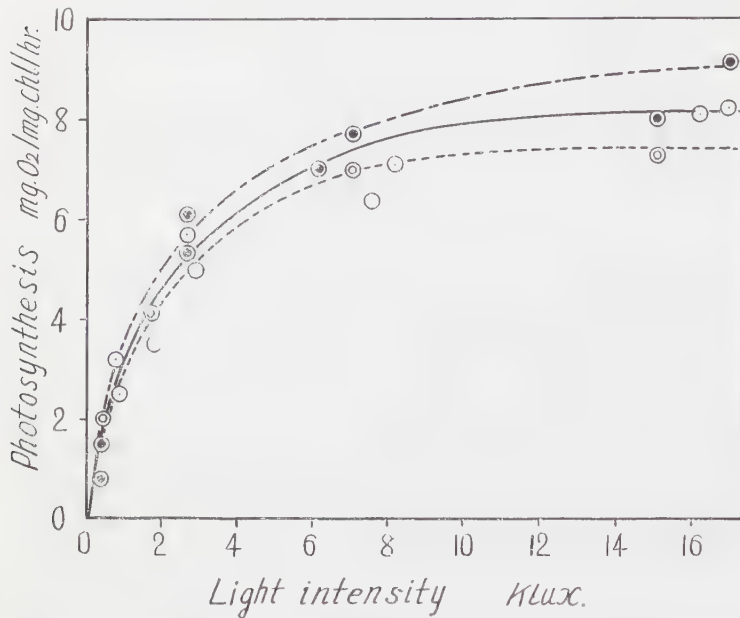


Fig. 1. Relation between photosynthetic rate and light intensity obtained with phytoplankton growing in waters taken from several eutrophic lakes.

--○--, *Microcystis* in Lake Kasumigaura, —●—, *Chlamydomonas* in Lake Jōnuma, -○-, *Fragilaria* in Lake Teganuma.

7 klux., a little lower than those obtained with two former samples.

The light saturation of photosynthesis was observed at low light intensity in the sample taken from the bottom of trophogenous layer. This is clearly seen in Fig. 2. The samples taken from various depths in Lake Kasumigaura in November included mainly *Microcystis* sp. The samples from the surface, and 2 and 3 metre depths showed nearly similar

Results of the experiment are shown in Fig. 1. From the feature of photosynthetic curves it may be said that the light saturation of photosynthesis somewhat differs with kinds of phytoplankters used. Namely, the light saturation occurred at about 15 klux. in *Microcystis* of Kasumigaura, and at about 10 klux. in *Chlamydomonas* of Jōnuma. However, that in *Fragilaria* of Teganuma was recorded

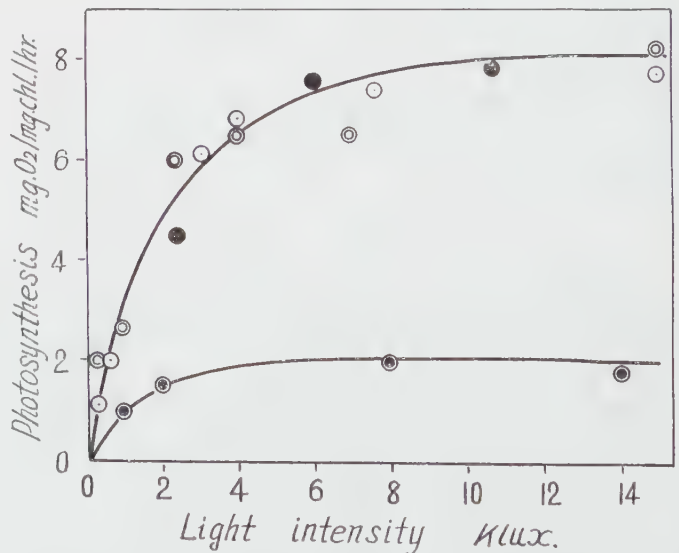


Fig. 2. Relation between photosynthetic rate and light intensity measured in phytoplankton taken from various depth (○ surface, ⊙ 2 m, ● 3 m, ⊖ 4 m) of Lake Kasumigaura.

reaction to light intensity with saturation at ca. 15 klux. From this result it may be concluded that the phytoplankton distributed in the trophogenous layer has the same photosynthetic activity, but the plankton living at the layer below the compensation depth has excessively low activity.

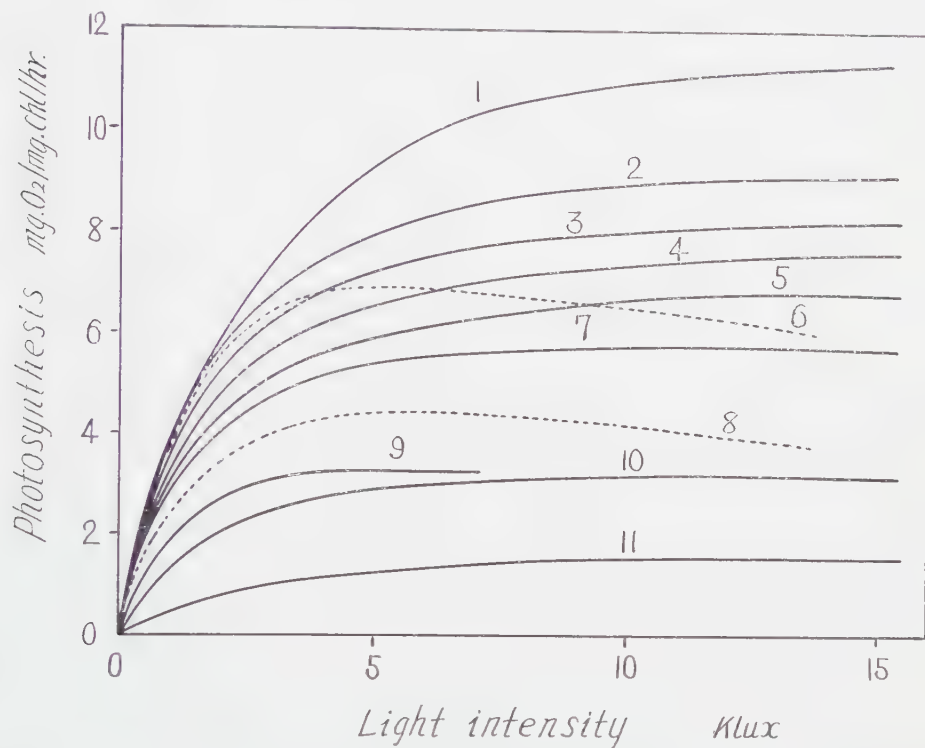


Fig. 3. Maximal photosynthetic activity of natural phytoplankton contained in raw waters taken from various waters.

1. *Chlorella* suspension, 2. Kasumigaura, 3. Jōnuma, 4. Nakanuma,
5. Teganuma, 6. Tokyo Bay, 7. Harunako, 8. Shimoda Bay,
9. Yamanakako, 10. Shojiko, 11. Ashinoko.

Phytoplankton collected from oligo- and mesotrophic lakes also showed the similar tendency in photosynthesis in relation to light intensity. However, the photosynthesis of these samples was, even of the surface water, already saturated with about 4 klux. light, and its maximum was strikingly lower than those of eutrophic lakes. For the low photosynthetic activity, in general, the deterioration of phytoplankton in deeper layer seems to be responsible in the above mentioned case, and the deficiency of nutrient salts does in oligotrophic lakes.

3. Photosynthetic rate of phytoplankton in various waters

The data of the photosynthetic rate of phytoplankton in natural water are very important in order to compare the primary production in lake with each other. In the previous paper (Ichimura 1958) the photosynthetic rate obtained under field condition

showed large divergency in each lake. In that experiment the accurate determination of light factor was too difficult to elucidate the relationship between photosynthesis and light intensity exactly. In this experiment, therefore, the photosynthetic rate was investigated under varying light conditions in a laboratory at constant temperature of 20°, and the nutrient conditions remained as they were. Sample waters taken from some eutrophic and oligotrophic lakes, the fundamental distinction of which had been disclosed by Yoshimura (1937) on the basis of nutrient materials in water. As

Table 1. Photosynthetic rate of phytoplankton in various waters, measured by ¹⁴C method.

Waters	Date	Photosynthesis O ₂ mg./chl. mg./hr.
Kasumigaura	May 16, 1957	9.1
Jōnuma	Oct. 29, 1956	8.2
Nakanuma	April 24, 1957	7.6
Teganuma	May 29, 1956	6.8
Harunako	Oct. 29, 1956	5.7
Kawaguchiko	June 28, 1956	4.6
Yamanakako	June 28, 1956	3.2
Shojiko	June 24, 1956	3.1
Ashinoko	May 23, 1958	1.4
Tokyo Bay	Mar. 20, 1956	6.8
Shimoda Bay	June 12, 1957	4.5
Kuroshio Area	May 2, 1958	2.0—1.5

compared with these waters,

Chlorella suspension in Detmer's solution, and sea waters were used.

The representative photosynthetic curves selected from the data obtained throughout the year are summarized in Table 1. The maximum photosynthetic rate

of phytoplankton is, in general, in eutrophic water about 8 mg. O₂/mg. chl./hr. and in oligotrophic water about 2—4 mg. O₂/mg. chl./hr. The curves, however, varied conspicuously according to the differences of water or of seasons. Such differences may be the result of the variation of nutrient concentration in water, and in some cases they may be attributable to the deterioration of phytoplankton itself. Details on this phenomenon will be discussed in the next paragraph relating to the effect of nutrient salts on photosynthesis.

4. Effect of nutrient salts on photosynthesis

The manipulation used in this experiment was similar to that reported by Edmondson (1947) as a whole. After two days keeping of sample waters of 20 l. added with various amount of Ca(NO₃)₂ and KH₂PO₄ in a growth chamber, with 10 klux. illumination at 20°, the sample waters were filled in clear and dark bottles and set out in a water bath at 20° in order to measure photosynthesis under varying light intensities. The effect of supply of nutrient salts on photosynthesis was exceedingly varied with the amount of those existing in the initial raw water.

Fig. 4 shows the results obtained with the surface water taken from extremely eutrophicated Lake Jōnuma in autumn, where the nutrient concentration was more than 0.2 mg./l. of NH₄-N and 0.08 mg./l. of PO₄-P (these analyses were carried out by

Dr. N. Yamagata, Gunma University). The amount of fertilizers added in 1 l. of enriched water were 0.3 mg. of nitrogen and 0.2 mg. of phosphorus. Photosynthetic rate of the raw water showed fairly high initial value, and the response of photosynthesis to fertilization was not so conspicuous. Marked response, however, was recognized with the sample waters from oligotrophic and mesotrophic lakes (Fig. 5). The surface water of mesotrophic Lake Yamanaka contained only about 0.01 mg./l. nitrogen and 0.002 mg./l. phosphorus in July 1956. There dominated *Fragilaria* sp. and *Melosira* sp. The photosynthetic

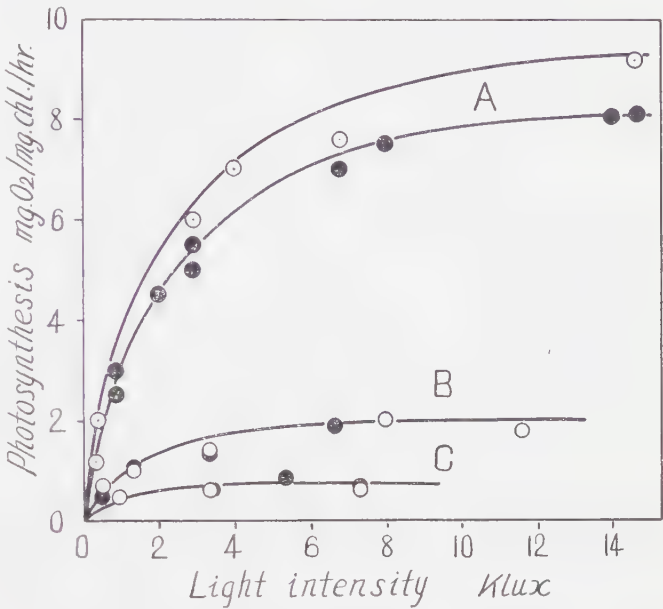


Fig. 4. Effect of fertilization with NO_3 and PO_4 on photosynthetic rate. Curves A: obtained with surface water taken from Lake Jönköping. Curve B: obtained with water taken at 4 m. depth of Lake Kasumigaura. Curve C: obtained with water taken at 1 m. depth of Lake Jönköping. Filled circles show the data obtained with raw water, and open circles those with fertilized water.

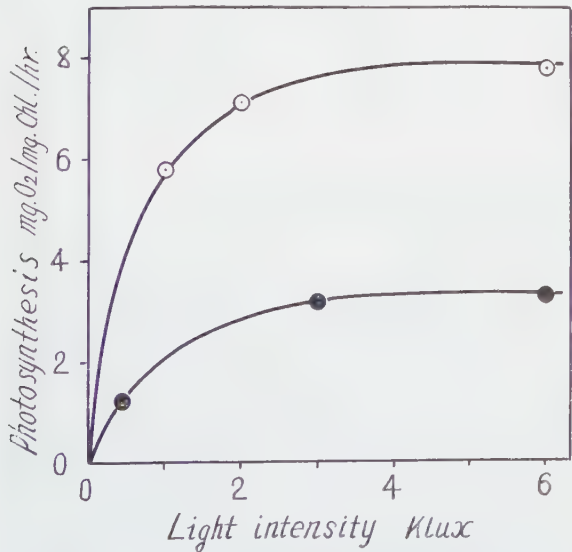


Fig. 5. Effect of fertilization on photosynthesis of phytoplankton living in surface water of mesotrophic Lake Yamanaka.
● Raw water, ○-enriched water.

activity of phytoplankton in such raw waters was very low and its maximum was less than 4 mg./mg. chl./l. With addition of proper amount of fertilizers ($\text{NO}_3\text{-N}$, 0.5 mg./l., $\text{PO}_4\text{-P}$, 0.2 mg./l.) the photosynthetic activity was promoted up to the high value which would be observed in eutrophic lakes. It may be inferred from these results that the low primary production generally measured in oligotrophic lakes should be referred to shortage of nutrient materials in water.

Depletion of nutrients frequently appears even in eutrophic lakes, generally in summer and in autumn.

As an example, the results obtained with samples taken from Lake Teganuma in October are summarized in

Table 2. Effect of nutrient salts on photoynthesis

Fertilizer mg./l.	Photosynthesis O ₂ mg./l./hr.	
	25°	15°
Raw lake water containing 0.01 NO ₃ N trace PO ₄ -P	0.16	0.12
Fertilized with 0.05 NO ₃ N 0.50 NO ₃ N	0.23 0.34	0.15 0.21
Fertilized with 0.05 PO ₄ P 0.50 PO ₄ P	0.29 0.39	— 0.22
Fertilized with 0.50 NO ₃ -N 0.50 PO ₄ -P	0.45	0.32

Table 2. Autumn peak of phytoplankton population which had generally been found in many lakes, was not observed in this lake. The amount of phosphorus in the water was almost nil, and only 0.01 mg./l. of nitrogen was detected. In this season application of fertilizers is quite effective on photosynthesis. Same phenomena were observed also in Lake

Kasumigaura. These results may suggest that one of the most important causes for autumn reduction of the primary production in lakes is rather the depletion of nutrients in water, and not due to the deterioration of phytoplankton.

The immediate effect of fertilization on photosynthesis was not recognized in the sample taken from deeper layer of lake. Lower curve in Fig. 4 shows the result obtained with sample waters from 1 m. depth of Lake Jōnuma and from 4 m. depth of Kasumigaura. The nutrient concentrations in the waters just after fertilization were 0.5 mg./l. of nitrogen and 0.2 mg./l. of phosphorus. In this case, the extremely low photosynthesis in the raw waters could not be recovered by fertilization. The cause is probably attributed to the deterioration of phytoplankton itself. However, it should also be taken into consideration that the phytoplankton living in feeble illumination of deeper layer sometimes is characterized by the photosynthesis of shade leaf type as measured by Gessner (1949).

5. Effect of temperature upon photosynthesis

The vertical diversity of temperature in trophogenous layer is not so remarkable as the decrease of light intensity with depth. Temperature factor rather seems to be important for determining the geographical and seasonal productivity (Ichimura 1954).

Experiments were done with waters collected from surface of several eutrophic lakes in every month. Tanks filled with sampled waters were incubated at various temperatures in out-of-door for 48 hours. Then, materials were filled into glass bottles and set out in water baths with various temperatures at which the sample had been incubated, with amplitude of $\pm 2^\circ$. Some results are indicated in Fig. 6. The productivity of water was generally increased with rising of temperature, although some of the samples which were taken in late summer or in winter did not show any sensitivity to temperature change. The results obtained with diatoms (*Fragilaria*, *Melosira*) and gree-blue algae are shown Fig. 7. The optimum tempera-

ture was about 15° in the former and 22°–25° in the latter. Diatoms showed more efficient activity than green-blue algae in low temperature range. However, the decrease of photosynthetic rate at high temperature is more noticeable in diatom than in green-blue algae. Moreover, it is interesting to notice that very high photosynthetic rate had sometimes been measured in field at about 5°, just after melting of ice, for example, in *Asterionella formosa* in Lake Harunako.

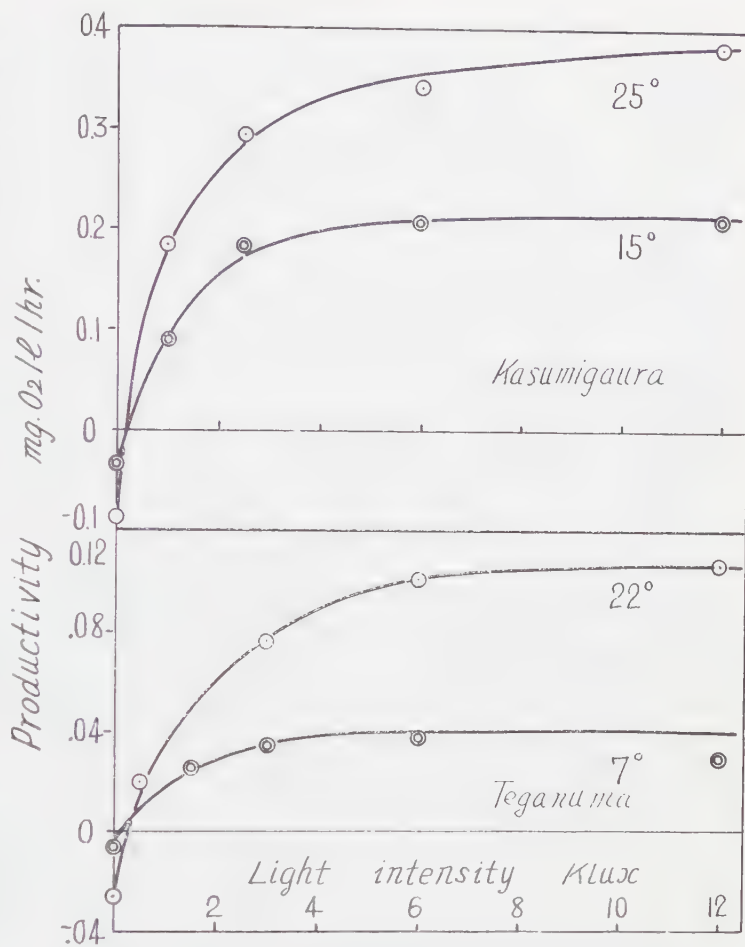


Fig. 6. Effect of temperature on productivity of surface water. Kasumigaura, Nov. 23, Teganuma, May 14.

6. Seasonal change in potential photosynthetic activity of phytoplankton

As shown in one of the previous papers (Ichimura 1958), the photosynthesis of phytoplankton indicates remarkable seasonal variation in the fields and variation is mainly attributable to the change of its environmental factors. However, there remains another serious problems whether phytoplankton living under severe natural conditions can keep its constant potential photosynthetic activity throughout the year. If the potential activity differs in each season, it would not be easy to deduce the primary production of a lake from simple calculation as introduced by Manning and Judy (1941), Hogetsu and Ichimura (1954), and Ryther and Yentsch (1957).

For the sake of elucidation of this problem, following experiments were carried out throughout the year at a constant temperature of 20° with lake waters supplied with nutrient materials in the optimal level of $\text{PO}_4\text{-P}$ 0.2 mg./l., $\text{NO}_3\text{-N}$ 0.5 mg./l. and

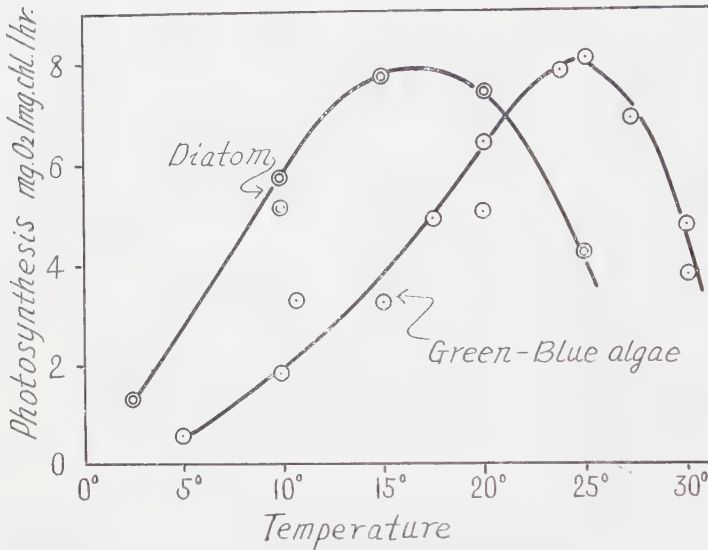


Fig. 7. Relation between photosynthetic rate and temperature measured in diatom and green-blue algae.

spells might be attributed to the deterioration of the phytoplankton. Actually, such spells accorded with the periods when the algae constituents were altering.

Summary

The relation of photosynthesis of natural phytoplankton to each environmental factor was discussed in detail on the basis of laboratory experiments.

1. In eutrophic lake water, or in water enriched with nutrient salts, light saturation of photosynthesis occurred at about 15 klux. in Cyanophyceae communities, at about 10 klux. in Chlorophyceae, and at 7 klux. in Bacillariophyceae. Such light saturation, however, was found at low light intensity of about 4 klux. with phytoplankton in oligotrophic lake water.

2. The highest photosynthetic activity of phytoplankton of about 8 mg. O₂/chl. mg./hr. was measured in natural waters of eutrophic lakes. The activity in mesotrophic lake was about 4 mg. O₂/chl. mg./hr. and the lowest was 2 mg. O₂/chl. mg./hr. in oligotrophic lakes.

tatol CO₂ 40 mg./l. The results obtained at light saturation are shown in Fig. 8. From the results it may be known that the potential photosynthetic activity of phytoplankton population is, as a whole, almost constant throughout the year, except for two spells in late summer and in late winter. Low photosynthetic activity in these two

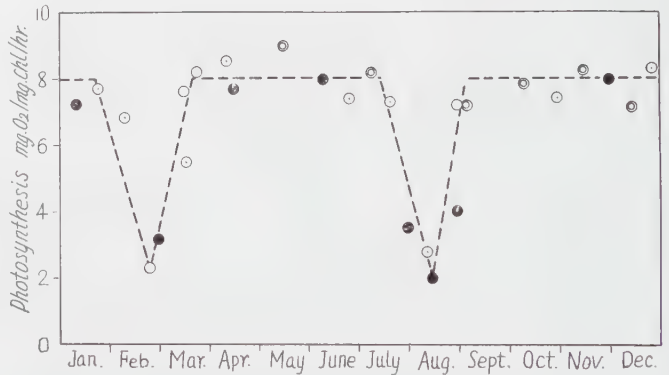


Fig. 8. Seasonal change in potential photosynthetic activity of phytoplankton of eutrophic lakes.

○ Lake Kasumigaura, ◐ Lake Teganuma, ● Lake Nakanuma

3. The effect of supply of nutrient salts on photosynthesis of phytoplankton was not so remarkable in eutrophic lake waters as in oligotrophic ones. However, nutrient materials in water were exhausted sometimes even in eutrophic water in summer and autumn, and the deficiency in such materials would be able to limit the primary production of lakes severely.

4. Potential photosynthetic activity of phytoplankton was almost constant throughout the year, except for two spells in March and in August when heavy altering of constituent phytoplankters were going.

The authors wish to express their cordial gratitude to Prof. M. Monsi and Prof. K. Hogetsu under whose guidance this research has been carried out. Also their thanks should be expressed to Prof. T. Miwa for his valuable suggestion and encouragement.

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Melanization in the Mycelium Due to the Interaction of Two Strains of *Neurospora crassa*

by Homare KUWANA*

桑名 誉*: アカパンカビの二系統間の相互作用による菌体のメラニン形成.

Received May 23, 1958

In the previous paper (Kuwana 1956), the author reported that tyrosinase activity of the homogenate of mycelium obtained from a mixed inoculation of two strains of *Neurospora crassa* is very strong, although the mycelial homogenates of the component strains show rather weak activities. In this paper, the author deals with the mechanism of the enhancement of tyrosinase activity and makes clear that it is due to the direct interaction by mixing of the cell contents of the two strains.

Materials

All strains used were derived from two wild type strains, 4A and 8a, of *Neurospora crassa*. From a cross of 4A \times 8a, two strains which produce abundant macroconidia were isolated; these are named K54A and K54a respectively. Either the mycelial homogenate of K54A or that of K54a showed little tyrosinase activity separately. But a mycelium obtained from a mixed inoculation of these strains showed a high tyrosinase activity (Kuwana 1956). From a further cross of K54A with K54a, eight complete asci were dissected. Four ascospore pairs from one of these asci named t2 were used exclusively. They are represented by t2-1A, t2-2a, t2-3a, and t2-4A. In these four isolates, the combinations between the same mating types gave strong tyrosinase activity.

Experimental

Hyphal fragments of the strains t2-1A and t2-4A were inoculated on two points on the surface of a Westergaard and Mitchell's (1947) agar plate and incubated at 25°. The two strains grow out and extend hyphae by radial growth. After five to seven days, the part of mycelium they met and to one side from this borderline became pigmented with melanin (Fig. 1). No such a mycelial melanization was found in the case where only t2-1A or t2-4A were inoculated at the two points (Fig. 1). The

* Department of Biology, Faculty of Science, Osaka University, Osaka, Japan. 大阪大学理学部生物学教室.

same phenomenon was observed with t2-2a and t2-3a. The strain t2-2a behaves as t2-1A, and t2-3a as t2-4A.

In order to gain the mycelium from the surface of an agar plate, a cellophane membrane was put on a Westergaard's agar plate, and t2-1A and t2-4A were inoculated at the two points on it. After seven days, the mycelium was harvested and cut to four parts as shown in Table 1, and the tyrosinase activity of the homogenate of each part was estimated by color reaction with the addition of L-tyrosine as a substrate. The enzyme activity was detected only from the part 3, melanized part of the mycelium (Table 1). The more accurate experiment was performed with the Warburg respirometer. In this case, activities of part 1+2 and part 3 were compared with each other. A strong tyrosinase activity was also detected only from the pigmented part (Table 1).

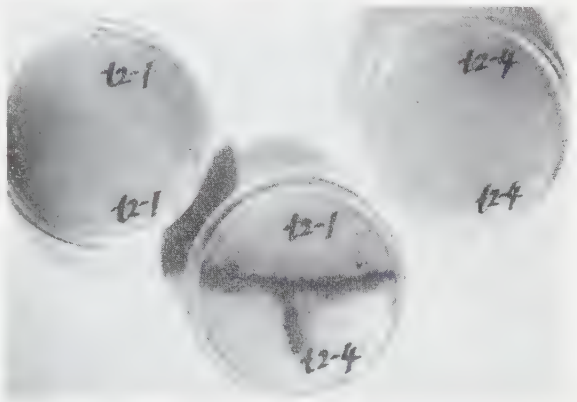
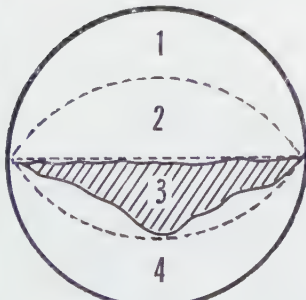


Fig. 1. The melanization reaction between the strains t2 1A and t2-4A, cultivated on the Westergaard's medium at 25° for 6 days.

Table 1. Tyrosinase activity of different part of the mycelium. The final concentration of the substrate, L-tyrosine, was M/600. Oxygen uptake was determined at 25°.

Partition of mycelium		Tyrosinase activity		
		Colorimetric estimation	Respirometric determination Q _{O₂} (μl./mg. wet weight/hr.)	
			Intact mycelium	Homogenate
	1	—	0.19	0.55
	2	—		
	3	+	1.37	11.45
	4	—		

Thus, a strong tyrosinase activity shown by the homogenate of the mycelium obtained from the mixed inoculation of two strains may be due to such a pigmented part.

Various environmental conditions affect the melanization of the mycelium as reported elsewhere (Schaeffer 1953, Hirsch 1954). Ten times as much concentration of the nitrogen source as the Westergaard's medium prevents the pigmentation. A temperature above 30° also prevents it. If the concentration of sulfur is reduced to one tenth, mycelial part of contact of the two strains shows the pigment even in the presence of the high level of nitrogen. These conditions coincide with those which enhance the activity of tyrosinase (Horowitz and Shen 1952, Schaeffer 1953, Hirsch 1954, Kuwana 1956).

A question is raised as to whether the melanization of the portion of mycelial contact of the two strains is due to diffusible substance(s) which are excreted from one strain and exert their effects on the other, or it is caused by the direct interaction by mixing of the cytoplasm of the two strains.

The first possibility was examined preliminarily with the use of U-tube, the base of which was partitioned by a cellophane membrane. The both sides of the tube were half filled with liquid or agar Westergaard's medium. The strains t2-2a and t2-3a were inoculated in each side respectively and incubated at 25°. No melanization was observed at the part of contact of both mycelia across the cellophane membrane even after three weeks. Tyrosinase activity was not high in the mycelial homogenates of t2-2a, or t2-3a, even when they were cultivated on the medium containing the culture filtrate of t2-3a and t2-2a respectively (Table 2). After the cultivation

Table 2. The effect of culture filtrates on tyrosinase activity. Filtrates were prepared from 7 days culture media through Chamberland bacterial filters. Tyrosinase activity of 5 days mycelial homogenates was estimated colorimetrically.

Medium Mycelium	Westergaard's (control)	Westergaard's + filtrate of t2-2a	Westergaard's + filtrate of t2-3a
t2-2a	—	—	—
t2-3a	—	—	—
t2-2a+t2-3a	++	+++	+++

in which the culture filtrates were exchanged between t2-2a and t2-3a on the way of incubation, tyrosinase activity was not high from their mycelial homogenate. The possibility of diffusible substances which act to increase tyrosinase activity, therefore, may be excluded.

On the other hand, the possibility of the direct interaction of the two cell contents through anastomosis to cause the melanization seems to be plausible. The fusion of

the hyphae of t2-1A and t2-4A were actually observed on a narrow strip of the Westergaard's agar on a slide glass by the method employed by Garnjobst (1956). After a minute or so, the part of hyphae fused were granulated and in nine out of ten cases these granules went toward the side of t2-4A (Fig. 2), which is to be pigmented. Although it could not be assured that the granulated part was actually melanized on the thin film on a slide glass, it seems probable that the melanization is the reaction caused by the direct mixing of the cell contents of the two strains.

In order to gain a clue to know the physiological significance of the melanization, heterocaryons were made between the two strains. Nutritional requirements were introduced by the irradiation

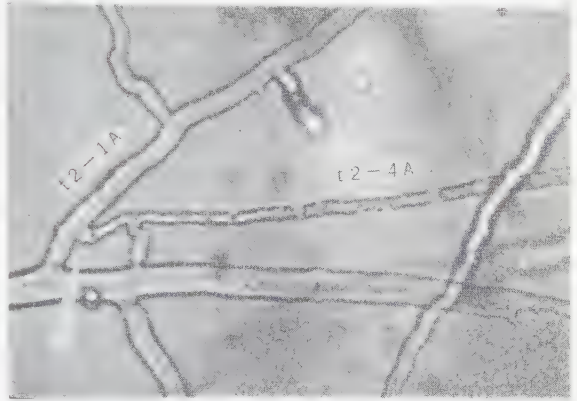


Fig. 2. Granulation of the cytoplasm in the hypha of t2-4A which fused with the hypha of t2-1A. The arrow shows the point of fusion.

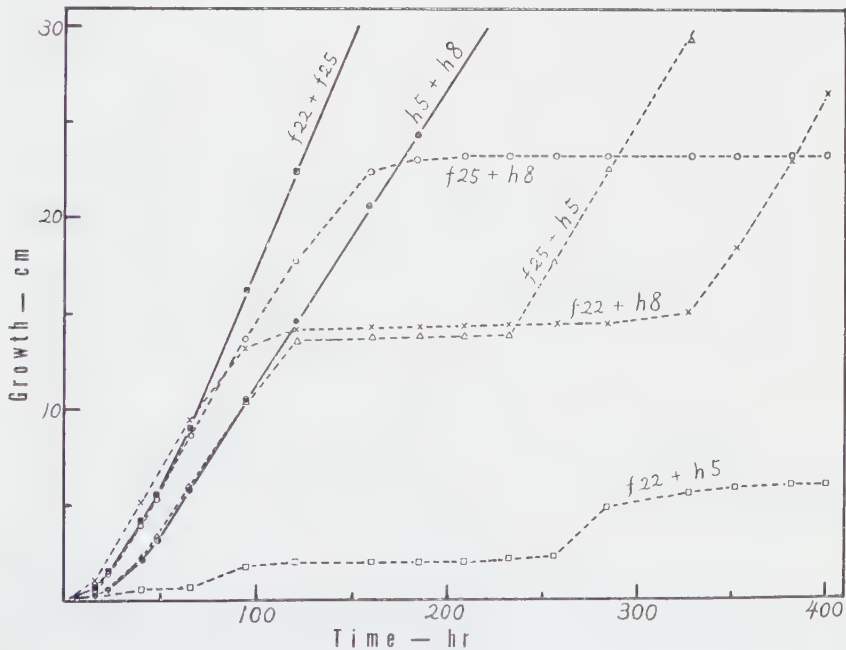


Fig. 3. Growth of heterocaryons. The strains f22 and f25 were derived from t2-1A, and h5 and h8 from t2 4A.

of ultraviolet light to the conidia of t2-1A and t2-4A. From the t2-1A, pantothenate-requiring (f22) and nicotinic-requiring (f25) strains were isolated and from t2-4A, methionine-requiring (h5) and nicotinic-requiring (h8) strains. The strains f25 and

h8 are not allelic because they gave heterocaryotic growth on a minimal medium. Heterocaryotic growth was determined by using the growth tubes described by Ryan *et al.* (1943). The results are shown in Fig. 3. The heterocaryons between the two mutants originated from the same strains, f22+f25 or h5+h8, grew as well as the original strains. But the heterocaryons between the two mutants originated from different strains showed irregular growth. Furthermore, the isolates obtained from the end of the growth tubes did not grow on a minimal medium. That is, the growing hyphal tips are composed of only one kind of component nuclei.

Discussions and Conclusions

As it is reported in the previous paper, if two wild type strains, which do not show a detectable tyrosinase activity separately were cultivated together in the same Westergaard's medium at 25°, the resulting mycelial homogenates in certain combinations gave very strong tyrosinase activity. In the experiments reported here, the melanization reaction between the two strains, carrying the same mating type, was visualized on the Westergaard's agar plate. That is, the part of mycelium where the two strains touch and one side from this borderline turned brown to black with melanin. And the homogenate of this pigmented part gave a strong tyrosinase activity. The evidence could not be obtained that either of these two strains excretes diffusible substance(s) which act to melanize the other strain. On the other hand, observations of hyphae showed that the cells of the two strains actually fuse and the cytoplasm is mixed. After about one minute, many granules appeared in the cells and expanded their presence in the mycelium. So it seems probable that the melanization and the high tyrosinase activity are due to the direct interaction by mixing the two cytoplasm in the common hyphal cell. The nuclei of the two strains, the combination of which gave a melanization reaction, repel each other, as shown in the heterocaryon experiment. It may be possible, therefore, that this incompatibility reaction causes the granulation and melanization of the cytoplasm.

Acknowledgment

The author expresses his hearty thanks to Prof. H. Kikkawa for his encouragement and good advice in the course of these studies.

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Karyological Studies in *Saccharomyces*

by Akira YUASA*

湯浅 明*: コウボキンの核学的研究

Received June 9, 1958

Though many investigations have been made on the nucleus and mitosis in *Saccharomyces*, they have not come to any conclusion. While Fuhrmann (1906) and Kater (1927) advocated mitosis, but, on the other hand, Guilliermond (1902) and Beams, Zell and Sulkin (1940) reported amitosis in *Saccharomyces*. The present author has already reported mitosis in *S. cerevisiae* incorporated with Sinotô, and thought that the haploid number of the chromosomes was four (1946).

Recently Subramaniam (1946, 1947, 1948, 1952 etc.) observed two big chromosomes in *Saccharomyces* and discussed actively against Winge (1951).

Lindgren (1950, 1951, 1956) and Lindgren and Rafalko (1950) observed spindle outside of the vacuole, in which centrochromatin was seen. When the mitosis began the centrochromatin went out into the vacuole and became chromosomes. So they recognized the vacuolar nucleus of Wager and Peniston (1910), but Royan (1956) confirmed the fact that vacuoles could not be seen in some cases.

Yoneda (unpublished) showed the nucleus of *Saccharomyces* by Feulgen's nuclear staining, and supposed four chromosomes in haploid besides the vacuole.

Recently many investigators have reported, using Feulgen's reaction or Giemsa's staining, and confirmed the true state of nucleus, but their opinions do not coincide. So it is necessary to criticize and bring various opinions into unity.

Materials and Methods

The materials studied are Line 8656 (haploid), 396, 7111, 7228, 7453 and 17807 (haploid). They are cultivated, using Lindgren's nutrient-solution, Ogur's solution and Czapek's sol. The cells were observed vitally or observed after fixation and staining. The fixatives used were Hilley's solution, Carnoy's fluid, Nawashin's solution or others. The staining solutions were aqueous solution of Gentian violet, iodide iodine, eosin, sudan III, haematoxylin, fast green, neutral red, toluidine blue, tetrazolium, Janus green, acid fuchsin and others. They were also stained by Feulgen's method or Giemsa's staining method. Perchloric acid was also used to take off RNA.

* Department of Biology, College of General Education, University of Tokyo, Tokyo, Japan.
東京大学教養学部生物学教室

Results

Nucleus

When stained with haematoxylin, a small body which has been called as centrosome by Lindegren (1952) is seen near the vacuole.

This body has been later called as spindle by Lindegren, Williams and McClary (1956) (Microphoto. 1a).

Lindegren and Townsend (1953) saw a crescent-formed centrochromatin in the spindle and lately described it as a star-formed chromosome (Lindegren, Williams and McClary 1956). In the present study when a yeast-cell was fixed with Carnoy's fluid, treated with perchloric acid (10%) and stained with Giemsa's method a centrochromatin was also seen in the nucleus (Fig. 1c). Sometimes the nucleus disappeared, leaving centrochromatin, or was stained lightly homogeneously. This showed that sometimes the ground substance of the nucleus was destroyed, leaving centrochroma-

tin only, or both the nucleus and centrochromatin were destroyed and the substance of centrochromatin diffused in the nucleus.

Sometimes the nucleus shows spiremes in its interior, according to the fixative and staining solution used (Fig. 1a, b). The status of the nucleus changes not only according to the fixative and staining method, but also the developmental stage.

But, by the Giemsa's method the portion of the centrochromatin other than DNA is also stained as deeply as DNA. So, by this method stained bodies are also seen in the vacuole. Sometimes Feulgen's stained bodies are also seen in the vacuole, but these bodies are thought to be aldehyde other than DNA.

In the vacuole there are often seen thread-like or bar-like bodies which show negative nucleal reaction. When the centrochromatin disappears during the course of nuclear division, however, there appears chromosome-like bodies in the vacuole which show Feulgen-positive nucleal reaction. So it is presumed that the round body which con-

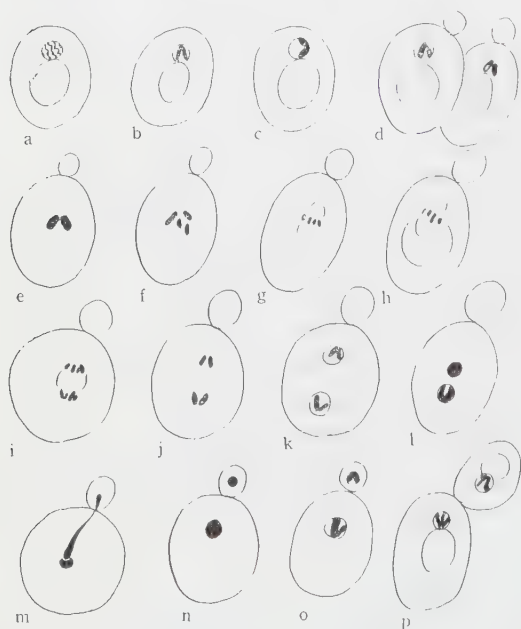


Fig. 1. The process of mitosis in *S. cerevisiae*, schematically shown. \times ca. 3,600. **a**, nucleus shows spireme in its interior. **b**, nucleus shows two chromatin-threads. **c**, nucleus shows centrochromatin. **d**, prophase. Two chromatin-threads are seen. **e**, metaphase. Two synaptonic chromosomes. **f**, metaphase. Four chromosomes. **g**, metaphase. Side-view. Chromosomes and spindle are seen. **h**, chromosomes, spindle and vacuole are seen. **i**, anaphase. Two chromatids are seen. **j**, telophase. **k, l**, two daughter nuclei. **m**, one of the daughter nuclei enters into the bud. **n, o**, the mother-cell and the bud have one nucleus, respectively. **p**, completed bud, having one nucleus and one vacuole.

tains the centrochromatin is the true nucleus and that the nucleus changes into chromosomes at the stage of prophase as reported by Lindegren (1945) and Lindegren, McClary and Williams (1955). But the chromosomes appear on the area of vacuole as if the latter contains the former. In the vacuole, however, there are thread-like or bar-like bodies which are stained with either Giemsa or haematoxylin (Microphoto. 1b, c).

Vacuole

In some stage of development the vacuole is not seen, but usually there is one large vacuole in the yeast cell. The yeast cell which shows no vacuole is also observed by Royan (1956) and Lindegren (1949).



Microphoto. 1. **a**, the so-called spindle (S) and vacuole (V) are seen. \times ca. 1,500. Fixed with 0.5% acetic acid. **b**, the so-called chromosomes (NV) in the vacuole. \times ca. 4,500. Fixed with Flemming's solution and stained with Giemsa's solution. **c**, true nucleus (N) and vacuole (V). Fixed with Flemming's solution and stained with Heidenhain's haematoxylin. \times ca. 3,000.

When stained with Giemsa or haematoxylin both the chromosomes and the thread-like or bar-like structure in the vacuole are all stained in the same place of the cell. So it seems that the chromosomes are also in the vacuole. The thread-like or bar-like structure in the vacuole, however, shows negative Feulgen's reaction.

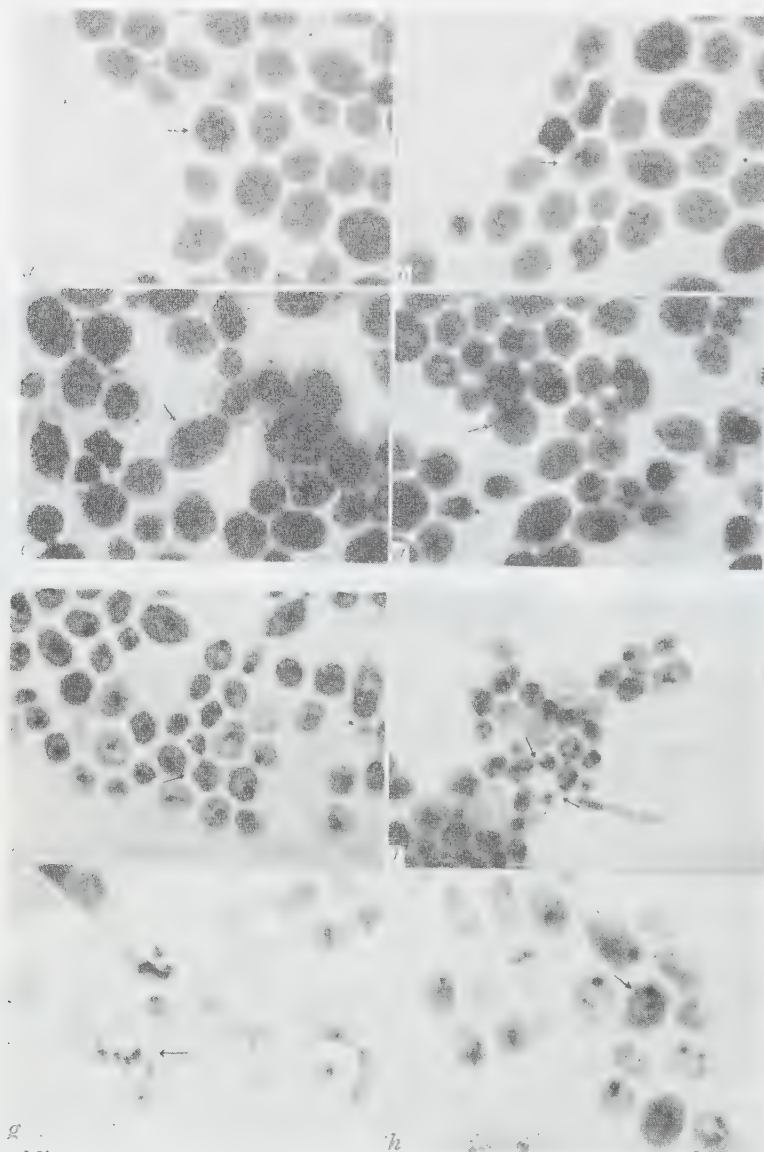
When stained with 1% aq. solution of neutral red several bar-like structures are seen in the vacuole and some of them are thought to be same with the structure which is stained with Giemsa or haematoxylin.

Stained with toluidine blue solution (pH. 11) bar-like bodies are seen in the vacuole. They are paired bodies as reported by Townsend and Lindegren (1953). Townsend and Lindegren (1953) thought them to be the crystalloid formed from toluidine blue.

Accordingly the present author thinks that there are three kinds of structures in the vacuole, namely; a) nucleolus-like body, b) toluidineblue stained body, c) neutral red stained body (some of them are also stained with Giemsa or haematoxylin).

Nuclear Division

With Giemsa's solution almost all the structures in the vacuole are stained and also the chromosomes are stained. The Feulgen's nucleal staining stains only chromosomes. In the case of Feulgen's staining, however, the vacuole is destroyed, so it



Microphoto. 2. **a-d**, fixed with Flemming's solution stained with haematoxylin. **e-h**, fixed with Carnoy's fluid, stained with Giemsa's solution. **a-d**, \times ca. 900. **e, f**, \times ca. 900. **g, h**, \times ca. 1,800. **a**, chromosomes are seen on the vacuole (arrow shows). **b**, spindle and chromosomes are seen. No vacuole. **c**, chromosomes spindle and vacuole are seen. **d**, two nuclei after mitosis are seen in the same cell. **e**, one of the daughter nuclei enters into the bud. **f**, the same with **e**. **g**, mitotic figure overlapped on the vacuole. **h**, chromosomes are distinct on the vacuole.

looks as if the chromosomes are contained in the vacuole.

At the beginning of the nuclear division the crescent-shaped centrochromatin changes into chromosomes and appears in the overlapping place of the vacuole. The various bodies in the vacuole stain, depending on the staining method. Therefore, at the first glance, it seems as if there are many chromosomes in the vacuole.

The centrochromatin changes, at first, into rod-shaped chromosomes and then four chromosomes in haploid line (Fig. 1d,f). Sometimes, however, four chromosomes appear, conjugating two by two (Fig. 1e).

After the nuclear division two nuclei are seen in a yeast cell, one of which enters into a bud. The ground substance of the nucleus which was called as spindle by Lindegren, Williams and McClary (1956) changes into spindle in which the chromosomes are seen, but the spindle is seen only with difficulties (Fig., g,h).

In some cases the spindle is seen clearly and at each pole of the spindle, however, there is no centriole which has also shown by Lindegren, Williams and McClary (1956). But, it may be seen in some cases by adequate techniques.

When one daughter nucleus enters into the bud, the other nucleus remains near the former as shown by Sinotô and Yuasa (1941). At the critical phase when the one nucleus enters into the bud, it shows a dumb-bell shape.

After the two nuclei have been completed, the spindle again becomes clearly and one portion of the spindle enters into the bud. By the aid of the spindle one daughter nucleus enters into the bud. After or before this process a new vacuole appears in the bud or some portion of the mother vacuole is divided into the bud.

The two daughter nuclei which have resulted from mitosis show homogeneous structures, but after one nucleus has entered into the bud every nucleus shows the crescent-shaped chromatin-material.

At the prophase of mitosis the centrochromatin changes into spireme-structure and sometimes two chromatin-threads (Fig. 1b). At the metaphase 4 chromosomes are seen in the nuclear area which overlapped on the vacuole (Fig. 1g,h). In this stage the spindle cannot be seen, but it appears again after two daughter nuclei have been completed and act to introduce one daughter nucleus into the bud.

In some cases only 2 chromosomes are seen in the metaphase of mitosis in haploid line. This is thought to be owing to the fact that four chromosomes conjugated two by two (Fig. 1e).

After mitosis one of the daughter nuclei enters into the bud and shows amitosis-like figure at that time (Fig. 1m). This fact has already been observed by Sinotô and Yuasa (1941) in *S. cerevisiae*.

Various figures of mitosis have been gotten, using various fixatives and staining solutions, but the principle of mitosis is same in all the cases.

The text-figure 1 shows the main features of mitosis in *S. cerevisiae* (ref. Microphoto. 2).

Discussion

In the resting stage of the yeast cell Lindegren (1952) observed vacuolar nucleus and centrosome which he (1956) later called as the spindle. In the spindle, Lindegren, Williams and McClary (1956) saw the chromatin material as network of small fibers or condensed rods. The present author saw crescent-shaped or rod-shaped chromatin in the spindle which showed positive nucleal reaction and was stained with Giemsa's stain somewhat thick, because Giemsa stained not only DNA, but also protein.

The present author thinks that the so-called spindle is the true nucleus in which centrochromatin changes into spiremes or two chromatin threads in prophase. At the metaphase, the ground substance of the nucleus changes into spindle as in the cases of higher plants. So the term spindle is not inadequate to call the true nucleus. The two chromatin-thread in the nucleus was observed by Townsend and Lindegren (1953) and Townsend (1956) showed a positive reaction of the true nucleus. According to Townsend and Lindegren (1953), focussing through the cell reveals that the centrochromatin is actually a cap covering part of the centrosome and maintaining contact with the vacuole.

The present writer saw chromatin-material in the nucleus, which is maldistributed to be crescent-shaped centrochromatin.

Lindegren, McClary and Williams (1955) studied on metaphase in the yeast-cell and observed volution-coated chromosomes which appeared purple without the filter by Lindegren's volution-stain. The size of the chromosome may be different according to the staining-method which stains various portions of the chromosome.

Summarizing the results of various investigations, Royan (1956) recognized the true nucleus outside of the vacuole. He also saw two chromatin-bodies in the nucleus, which were also observed by the present writer.

As stated above the size of chromosomes is different according to the method of fixation and staining, so the figure which is called chromosomes is different according to the study-method. The two chromosomes which have been observed by Subramaniam (1951) are thought to be the chromosomes which are composed of Feulgen-positive chromatin material and of the other protein. Winge (1951) opposed against the Subramaniam's idea.

At the stage of mitosis the nuclear membrane disappears and chromosomes which are Feulgen positive appear. At the same time the vacuolar membrane often disappears, so the chromosomes look as if they exist in the vacuole. The chromosomes which have been observed by Lindegren, McClary and Williams (1955) are thought to be the conjugated structure of the true chromosomes and the intravacuolar bodies.

The number of chromosomes is four in haploid phase. Sometimes four chromosomes conjugate and become two. They show Feulgen positive reaction. The two conjugated chromosomes are recognized also by Subramaniam (1952).

In the vacuole there are various bodies which are stained by various staining

dye solutions. Some of them are stained with toluidine-blue solution and some with neutral red solution. Sometimes true chromosomes are stained together with the intravacuolar bodies. In this case it seems that there are many chromosomes in the vacuole.

Summary

1. The vacuole appears according to the condition in which the yeast-cell exists.
2. In the vacuole there are nucleolus-like body, toluidine-blue stained bodies and neutral red stained bodies. They are recognized when adequate staining method is used.
3. The nucleus is attached to one side of the vacuole and contains centrochromatin which changes into chromosomes in mitosis.
4. At the prophase of mitosis the centrochromatin changes into two chromatinic bodies. Sometimes the nucleus shows spireme in its interior. At metaphase the nucleus changes into four chromosomes which sometimes conjugate two by two.
5. At the metaphase the chromosomes appear in the spindle which is situated on the surface of the vacuole. So the chromosomes overlap on the intravacuolar bodies.

The writer wishes to express his cordial thanks to Mrs. Masako Osumi, Miss Hiroko Nakamura, Miss Akiko Kubo, Miss Kyoko Omura and Miss Reiko Nasu for their assistance throughout this study. Thanks are also due to Dr. Hiroshi Iizuka, Institute of Applied Microbiology, University of Tokyo, and Mr. Takeshi Tabuchi, Faculty of Agriculture, Tokyo University of Education, who kindly supplied the materials.

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On the Artificial Pollen Grain Germination of *Zea Mays* L.*

by Atsushi KUBO**

久保 淳*: トウモロコシ花粉の人工発芽について*

Received March 12, 1958

It has generally been known that the pollen grains of Graminae^{2),13),16),20)}, as well as those of Ericaceae and Compositae^{6),7),9),16)}, do not germinate well on any of artificial media. In *Zea Mays*, however, there are some reports; by Pfundt¹¹⁾ 20~30% germination on 10~30% sucrose-0.7~1.0% agar medium, by Andronescu¹⁾ 20%, by Sasaki¹⁴⁾ 4.9%, by Knowlton⁴⁾ 6.2%, by Tabata¹⁵⁾ 49.7~62.6% and by Gotoh²⁾ 55.0%. Gotoh²⁾ exposed pollen grains for 1.5 hours in the air and obtained better result of 89.3%. But the author was less successful in the same methods as theirs. Nakayama¹⁰⁾ maintained that the results by Tabata¹⁵⁾ and Gotoh²⁾, who had taken swollen proboscides as an early stage of pollen tube development, might not be enough for the observation on Graminae pollen, because in these plants the appearance of swollen proboscides did not indicate germination. Whatever plants the author might study, he counted only the pollen grains with developed pollen tubes as germinable ones and excluded the ones with swollen proboscides. On 20 μ thick layers of gelatin medium which could control quantity of water suitable for pollen germination^{5),6),7),8),12),13),18)}, the author observed high germination rates in the pollen grains of Ericaceae, Compositae and *Triticum vulgare*^{6),8)}. So the author tried to germinate pollen of *Zea Mays* on the 20 μ thick layers of gelatin medium. Results are in the following.

Material and Method

The native common flint corn was cultured in the field of Fukuoka Liberal Arts College in 1954. About thirty seeds were sown monthly from March to November. The earliest cultured plants flowered in July. The germination experiments of pollen grains were carried out from that time to the end of the year.

Experimental results

In the morning fresh pollen grains were collected in the field, kept in Petri dishes and were sown on the layer of medium.

Exp. 1. On agar medium. On agar medium the author could not get good results enough to be denoted in a table. During Oct. 4~18 (av. temp. 19.6°) the average rates

* The problem of physical and physiological dryness. Report 21. By Y. Fukuda

** Botanical Institute, Faculty of Science, Hiroshima University. Hiroshima, Japan.
広島大学理学部植物学教室

of germination in 6 days were 7.2~17.9% (av. 12.4%) on lean agar of 0.5~3.5%, and 20.6~32.1% (av. 24.6%) on agar with 5% sucrose. Variation of agar concentration did not affect the germination, even though addition of sucrose was slightly effective. On some occasions in October no pollen grains germinated, while on other occasions as high as 40.3% of them germinated. Such high record was not obtained before October. In December no pollen grains germinated.

Exp. 2. On gelatin (manufactures of Yasu Co.) medium. During Oct. 4~14 (Table 1) on the 20μ thick layers of 10~40% gelatin the highest germination rate was 79.5% (av. 50.6%). Addition of sucrose was fairly effective on the germination: with 5% sucrose the highest germination rate was 89.1%. On thicker layers any grain was hardly able to germinate; the good results were obtained only on the 20μ thick layers. The 20μ thick layers of 20~30% gelatin with 5~25% sucrose, gave specially good results.

Table 1 Germination percentages on sucrose-gelatin of wide concentration ranges.

Concentration of sucrose	Date and air temperature					av.	Gelatin concentration
	Oct. 4 21°	5 20°	7 19°	10 18°	14 22°		
0 %	77.9	79.5	59.7	31.7	4.3	50.6	10~40 %
5 %	87.6	89.1	86.9	55.9	8.8	65.7	
5~25%	94.2	88.0	63.6	56.1	22.6	64.9	20 %
5 grades	99.6	98.6	70.9	40.6	7.6	63.5	30 %
av.	89.8	88.8	70.3	46.1	10.8		

As denoted in the Table 2, 100% or special good germinations were recorded on Oct. 4 and 5.

Table 2 Do. on the good medium in October.

Concentration of gelatin	Date	Concentration of sucrose (%)					av.
		5	10	15	20	25	
20 %	Oct. 4	89.3	100	81.9	100	100	94.2
	Oct. 5	64.5	92.7	100	87.5	95.3	88.0
30 %	Oct. 4	100	100	100	100	98.0	96.6
	Oct. 5	100	98.0	95.0	100	100	98.6

As shown in Table 1, germination was more difficult in the middle of October (10.8%) than in the early October (89.8%). On Nov. 29 the results became worse as shown in the Table 3.

Table 3 Do. on the good medium on November 29.

Gelatin %	20						30					
Sucrose %	25	20	15	10	5	av.	25	20	15	10	5	av.
Lab.(air temp. 15°)	16.0	28.1	10.0	0.1	0	10.8	68.6	12.8	15.4	31.2	6.0	26.8
Green house (20°)	8.5	2.2	0.1	0	0	6.1	45.1	72.6	6.0	38.7	18.0	36.1
Incubater (24°)	10.8	6.1	0.1	0	0	3.4	7.3	12.2	0.8	7.8	3.6	6.3

The author, however, obtained 100% germination by increasing the amount of sucrose in the medium up to 40%. On the medium of 30~35% gelatin with 25~40% sucrose, 100% germination was observed.

Table 4 Do. on the best medium on December 1.

Gelatin %	30				35				40					
Sucrose %	40	35	30	av.	30	25	20	av.	25	20	15	10	5	av.
Lab. (air temp. 14°)	100	96.0	78.5	91.5	100	100	93.8	97.9	3.5	2.8	9.7	28.1	1.6	9.1
Green house (20°)	100	100	90.7	96.9	76.4	63.6	98.2	79.4	23.6	12.8	26.5	18.3	0	16.2
Incubater (24°)	3.8	6.5	4.3	4.9	6.0	26.3	12.1	14.8	4.8	9.8	4.5	2.6	0	4.3

After that severe weather damaged pollen grains and no pollen grains germinated even on this best medium.

Exp. 3. Effect of pH. The buffers used for the experiments were prepared by mixing the following solutions: M/25 of phosphoric acid, acetic acid, boric acid, respectively, and N/5 sodium hydroxide. The results are shown in Table 5.

Table 5 Do. with buffer reagent.

Date	Oct. 4	9, 10, 18	4	7	12	16
Medium pH	Agar 1.8% Sucrose 5%	Agar 2% Sucrose 5%	Gelatin 4% Sucrose 5%		Gelatin 20% Sucrose 20%	
2.8	4.0	0	0	0	0	0
3.8	0	0	0	0	0	0
4.8	0	0	17.3	0	0	0
5.8	0	0	0	0	0	0
6.8	0	0	0	2.3	0	0
7.8	0.1	0	0	18.5	0	0
8.8	4.3	0	1.0	33.6	0	0.7
9.8	5.2	0	10.0	0.1	0	1.0
10.8	12.5	0	5.7	0	0	0
11.8	0	0	6.7	0	0	0

When the medium containing buffer solution the germination rates were low either on agar or on gelatin even when sucrose was added. In the previous experiment 20% gelatin containing 20% sucrose was excellent, but in this experiment the buffer solution made this medium unsuitable for germinating. The higher the concentration of gelatin medium became, the more the pollen germination was inhibited. That is, as the water content in medium became less, the germination will be inhibited more strongly by ions. Both agar medium and gelatin medium were somewhat better in alkaline reaction, because under this condition the colloidal medium will swell well and get high swelling pressure^{12),17),19)}. The rise of swelling pressure of the medium, as well as the addition of concentrated sucrose, may effect better on germination of this pollen.

Conclusion

The author studied the pollen germination of *Zea Mays* from July to December.

The experiments in the summer did not bring good results, though the procedure described in the previous chapter was employed at that time. So no description was given in this paper. Just as it became autumn, the pollen grains produced at that season germinated on the artificial media.

The best media were found as follows:—

In October		In November	
Gelatin	20~30%	Gelatin	30~35%
Sucrose	5~25%	Sucrose	20~40%

As the season became later the pollen grain germinated on the more condensed and stiff medium. It suggests that pollen grains become more strongly xerophilous as the season become later. Therefore, it may be considered that the pollen grains produced in summer are not xerophilous. So they may germinate on dilute and soft medium. Device by the author seemed to be not enough suitable for germinating such sensitive unxerophilous pollen grains.

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本 会 記 事

北 海 道 支 部

第 11 回大会 (6 月 14 日, 於北大・理)

伊藤浩司: アツケシソウの群落。斎藤 優・宇佐美正一郎: 酵母のフェノール酸化について。中平雅子: 数種の人体寄生菌のアミノ酸々化。菅原繁蔵, 松川昌弘: スギナ類についての一考察。増淵法之: 秋まき性コムギにみられた分枝性異常穂について。細川久仁彦: タマネギ茎内の生長物質。辻 寧昭: 日本新産 *Callocolax* の一種について。酒井 昭: 超低温における植物組織の生存。沢田義康: ザゼンソウ, ミズバショウの花粉の発芽におよぼすアミノ酸の影響について。〔特別講演〕倉林正尚: 集団遺伝学。時田 郁: 欧米をめぐりて。

関 東 支 部

5 月例会 (5 月 24 日・於東大・理・植物)

原 襄: 葉縁生長について。門司正三: 西ハキスタン事情。

中 部 支 部

第 51 回例会 (5 月 17 日, 於名大・教養・生物)

堀田康雄: シダ前葉体の形態分化と生長物質。山高 桂: 変形アサガオについて。なお同支部の幹事にはあたらしく藤井良平, 高尾昭夫の両氏があたられます。

北 陸 支 部

第 30 回例会 (6 月 28 日, 於福井大・学芸学部)

鈴木米三・南茂睦隆: 黄色ルピナスのめばえによる L-Lysine の酸化について。河合 功: 蘚類の水分生態学的研究。小野寺正二: トチカガミ表皮の原形質分離形。

なお同支部の役員は次のように決定しました。

支部長: 正宗敬敏, 庶務幹事: 河合 功。会計幹事: 玉井直人。図書幹事: 里見信生。地方連絡幹事: 鈴木米三, 香室昭円

近 畿 支 部

昭和 32 年度第 3 回例会ならびに総会 (6 月 1 日

於京大・理・植物) 高木虎雄: タケザサ科の実生について。村田茂三: 茶葉の滲透圧の日変化について。上野実朗: バリの緑地帯

九 州 支 部

第 8 回支部大会 (5 月 24, 25 日, 於九大・理・農) 清水正元: 外界の温度変化と種子の寿命。岡岡 行・小島 均: 物質蓄積におよぼす地温の影響。宇佐美和夫: ワックス処理が植物切り枝の水分関係におよぼす影響。吉岡俊三: 挿子の頂芽の有無と再生成積ならびに滲透圧との関係。二宮淳一郎: 結合水含量と原形質粘土との関係について。稲田朝次・吉武哲夫: アラセイトウの花色について。楠元 司: 奄美大島の常緑広葉樹の光合成について。千葉保胤・菅原 淳: 精製葉緑体のリボヌクレアーゼの活性について。山根銀五郎・東 四郎: マメ科植物の根のアミノ酸の分泌について。頼綱理一郎: 植物体内生態学の理論と実際。新 敏夫: 琉球列島の蘚類II。尼川大録: 南アルプスの苔類相。三宮正信: コナラ属 3 種の染色体。藤原 勲: オオバコ属の核型分析。野田昭三: ツルボの分析, 核型およびその変異。井上 寛: 九州産 *Karimeris incisa* 群の細胞学的研究 I。染色体数と地理的分布。三宮正信・木梨満智子・鈴木時夫: イタビ類の偏心的肥大生長。二村和八・井原伸芳: シイ型およびウバメ型の森林における生活のリズムと一般気候との関係。奥富 清: 向字品のシイ群落の発達について。鈴木時夫・真柴茂彦: 九州中部山岳植生の組成群 I。带状森林植生。鈴木時夫・阿部泰雄: 九州中部山岳植生の組成群II。非带状草原植生

第 50 回例会: (6 月 21 日, 於福岡高校)

宮田逸夫: 蘚類の孢子発芽の茎葉再生について。梅埜国夫: ネナシカズラにおける色素形成について。

第 5 回熊本例会 (6 月 21 日, 於熊本大・図書館)

渡辺綱男: サツマノギクの細胞学的地理学的研究。山城 学: 熊本県産植物数種について。沢田耕尚: 暖地飼料作物の生育経過と品種との関係に関する研究。

新 入 会 員

(昭和 33 年 1 月—昭和 33 年 6 月)

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おしらせ

先月号の投稿に関する記事の一部を次のように変更いたします。

- 1) 欧文原稿には和文摘要(400字詰め原稿用紙2枚以内)をつけ、和文題名を明記してください。

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Ecological and Physiological Studies on the Vegetation of Mt. Shimagare

I. Preliminary Survey of the Vegetation of Mt. Shimagare

by Yasuyuki OSHIMA*, Makoto KIMURA*, Hideo IWAKI**
and Sumio KUROIWA**

大島康行*, 木村元*, 岩城英夫**, 黒岩澄加**: 縞枯山の植生に関するの
生態学ならびに生理学的研究 I. 縞枯山の植生の予備調査

Received May 21, 1958

Mt. Shimagare (2395 m. above the sea) is one of the hills in the northern part of Yatsugatake dead volcano group, of which the highest peak is Mt. Akadake (2899 m.), in Nagano Pref., at a distance of 130 km. NWW from Tokyo. In the dark green of the gentle southwest slope of Mt. Shimagare covered with subalpine coniferous forest, several whitish stripes horizontally running in parallel with each other can be seen in a distant view so distinctly that the mountain has been named "mountain with dead trees strips" according to its conspicuous physiognomy (Photo. 1, 2 and Fig. 1). The vegetation consists of several forest units, each of which has a serial arrangement from very young tree offspring in higher part up to overmatured and dead trees of *Abies Veitchii* and *A. Mariesii* in lower part, just as the natural image of Wagner's border cuttings¹⁾ (Fig. 2). The whitish stripes are the dense stands of dead tree trunks of these conifers (Photo 3), and they will shift upwards in decades.

Okubo¹⁰⁾, Yoshida and Yamanouchi¹²⁾ have already surveyed the forests from the view-point of forestry. They collected a lot of preliminary informations on the size, density and growth of trees, and tried to discuss the origin and shifting of the dead trees strips, though rather speculatively. Therefore, these problems might be well worthy of more precise ecological and physiological investigations into the origin and maintenance of such forest unit arrangement, especially on the basis of the dry matter production¹⁾ of the trees, because these investigations will bring many new findings about natural features of intra- and interspecific competitions and of plant succession, of which fundamentals have been studied theoretically and experimentally by the authors and their co-workers^{4), 5), 6), 7)}, and about the climax vegetation in the subalpine of central Japan.

* Department of Biology, Faculty of Science, Tokyo Metropolitan University, Setagaya, Tokyo, Japan. 東京都立大学理学部生物学教室

** Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan. 東京大学理学部植物学教室

In this first report, the results of the preliminary survey of the vegetation in 1957 will be discussed, referring to the papers of Okubo and of Yoshida and Yamanoichi before entering further the ecological and physiological studies on the vegetation and on the growth of the trees.

1. General features

The subalpine coniferous forests of Mt. Shimagare mainly consists of *Abies Veitchii* and *A. Mariesii*, being mixed with *Picea jezoensis* var. *hondoensis*, *Betula Ermani* and *Sorbus* sp. Such vegetation has been nominated by Nakano after his detailed studies on the vegetation of Mt. Yatsugatake and others, as *Betuleto Ermani-Abietetum Veitchi* association of a wide distributing *Betulion Ermani* alliance in subalpine coniferous forests in Japan. More strictly the vegetation of Mt. Shimagare will chiefly be classified into *Pteridophyllosum* sociation characterized by an obligate shade plant *Pteridophyllum racemosum*, of a subassociation *Mariesetum* in the association. On the southwest slope (10° – 20°) where the dead trees strips develop, the forests seem to be rather a consociation of the two *Abies* species, as *Betula* and *Sorbus* are scarcely mixed in the forests. The *Abietum* on the northeast slope (20° – 25°) has a considerable number of old trees of *Betula Ermani* and other deciduous species mixing.

Temperature and precipitation in the region of Mt. Shimagare will be able to assumed from the meteorological data of Kirigamine (1925 m. above the sea, 12 km. NWW of Mt. Shimagare) in Table 1. The temperatures at 2250 m. above the sea (the lower limit of the dead trees strips) and 2395 m. (the top of the mountain) have been calculated with lapse rate of temperature between the two stations (mean lapse rate was ca. 7.1° against 1000 m. elevation).

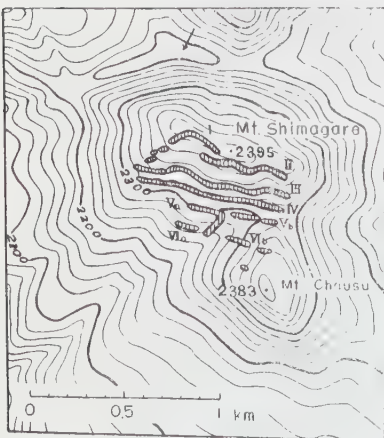


Fig. 1. Map showing the location of "dead trees strips" at Mt. Shimagare, which are corrected with the aerial photo of Geographical Survey.

The temperature climate at Mt. Shimagare is nearly same as that of Poronisk in Saghalin ($49^{\circ}12'N$; min. -17.5° in Jan., max. 15.9° in Aug., mean ann. temp. 0.0°). The precipitation at Mt. Shimagare seems to be same as or probably somewhat higher than that of Kirigamine because of higher altitude, and this amount may be enough or sometimes too much for tree growth under such low temperature conditions, comparing to that the annual precipitation at Poronisk is only 750 mm., at Quebec in Canada 1073 mm. (mean ann. temp. 3.6°), and at Davos in Switzerland 994 mm. (1561 m. above the sea; mean ann. temp. 2.8°).

Throughout the year west wind prevails on this slope of the mountain^{10),12)} causing



Photo 1. Mountains of the northern part of Yatsugatake. Left to right are Mt. Yokodake (2473 m.), Mt. Shimagare (2395 m.) and Mt. Chausu (2388 m).

Photo 2. Subalpine coniferous forest on the southwest slope of Mt. Shimagare, showing the "dead trees strips"



Photo 3. "Dead trees strips" IV with thick vegetation of *Abies Veitchii* and *A. Mariesii*.

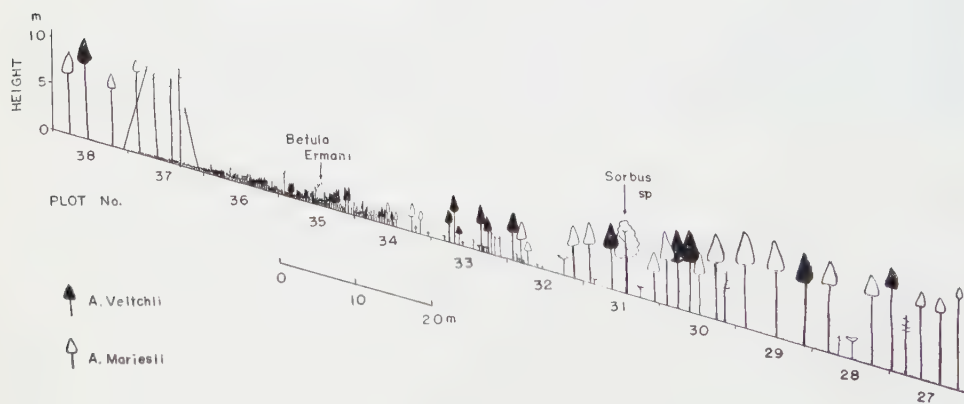


Fig. 2. Profile chart of Forest Unit IV on Transect I (West).

Table 1. Temperature and precipitation in the range of Mt. Shimagare. The temperatures at 2250 m., and 2395 m. are extrapolated from the data at Kitayama and Kirigamine.

Altitude	Kitayama		Kirigamine		Shimagare	
	985 m.		1925 m.		2250 m.	2395 m.
	mean air temp. (1916~40)	precipitation (1897~1944)	mean air temp. (1944~46)	precipitation (1944~46)	mean air temperature (calculated)	
Jan.	-3.2°	41 mm.	-10.5°	23 mm.	-13.0°	-14.2°
Feb.	-2.7	57	-10.7	59*	-13.5	-14.7
March	1.2	73	- 6.3	89	- 8.9	-10.1
April	8.0	104	0.9	130	- 1.6	- 2.7
May	12.7	103	5.8	136	3.9	2.4
June	17.0	172	11.3	194	9.3	8.4
July	21.1	178	14.4	240	12.1	11.1
Aug.	20.6	117	15.7	167	14.0	13.3
Sep.	17.8	119	11.6	176	9.5	8.5
Oct.	11.4	120	6.3	283	4.5	3.8
Nov.	5.9	69	0.5	88	- 1.4	- 2.2
Dec.	0.2	46	- 8.6	99	-11.6	-13.0
Annual	9.2°	1295 mm.	2.5	1683 mm.	0.2°	- 0.8°

*Mean precipitation in 1945~47; abnormally high precipitation of 579 mm. was recorded in 1944.

a slight deformation of tree crowns, and high humidity can be expected for the sake of thick fog which usually envelops the mountain.

The soil of the forest permits only shallow root development of the trees in a thin layer of brown loam of 30-40 cm., which is covered with mosses, debris of needles and humus. Under this loamy layer there exists a layer of large gravel up to 60-80 cm. depth on unweathered andesite rocks. Any speciality of substratum was not recognized at the dead trees strips (cf. also Yoshida and Yamanouchi 1955).

2. Structure of the forests and the dead trees strips

As mentioned above, the *Abies* forest on the southwestern slope of Mt. Shimagare can be separated into several forest units which are imbricating with each other. A forest unit consists of young tree growth in the upper part, of mature trees in the middle, and of overmatured ones in the lower part, ending in a dead trees strip. About six dead trees strips (Fig. 1), of which width is ca. 10 m., and length 300-800 m., are running on the slope along contour lines, almost in parallel with each other at intervals of about 100 m. The lowest dead trees strip (VI) situates at the 2250 m. above the sea, and a part of the highest strip (I) reaches the mountain top of 2395 m. and these areas fall in the natural distribution range of these *Abies*, as Imanishi²⁾ reported that the distribution range of *A. Veitchii* in the northern

Japanese Alps is 1636–2337 m. and that of *A. Mariesii* 1965–2550 m. The mixing proportion of *A. Mariesii* increases gradually towards the mountain top, at the cost of decreasing of the number of *A. Veitchii*.

In order to study the structure of the forest unit more precisely, two transects of about 250 m. long were laid down along the southwest slope, with an interval of 30 m., across the middle of the dead trees strips III, IV and Va. Quadrats of 1 m. \times 1 m. were employed at the younger tree stands because of dense and fairly homo-

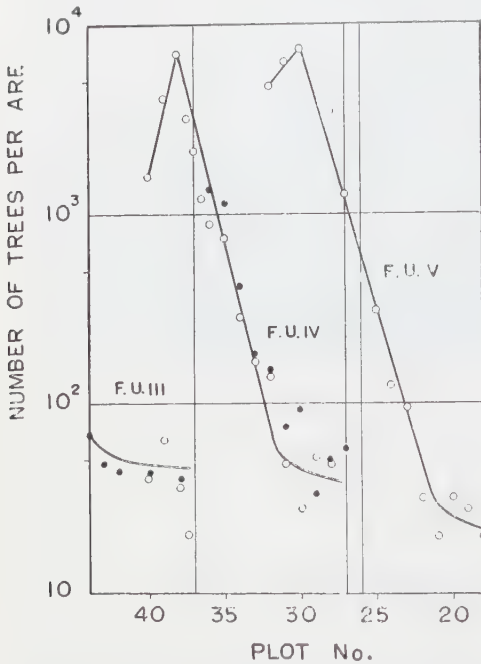


Fig. 3. Change of tree densities against descending the slope. Open circles show the data from Transect II and filled ones those from Transect I. Vertical lines correspond to the location of "dead trees strips" III and IV.

the decrease of number of trees. At the dead trees strips there occurs the regeneration of young thick growth of *Abies Veitchii* and *A. Mariesii*. This young mixed growth continues beyond the dead trees strip upwards to the seedlings under the canopy of the upper forest unit, as the germination of *Abies* trees and the establishment of the seedlings are going under the matured *Abies* trees at the in-between of the dead trees strip and another upper one. Downwards out of the forest canopy, the trees increase their heights and ages but decrease their density, forming a new lower forest canopy with mature and overmature trees. Then comes another or lower dead trees strip. The number of young growth increased in the seedling and very young tree stage with increase of plant age or downwards on the slope (see Fig. 3 and Tab. 2). After reaching the highest density at somewhat high above the upper dead

genous growth, but in the older tree stands, branch transects of 5 m. \times 100 m. perpendicular to the main transects or parallel to contour lines were adopted to get average values from the forest stands where the mature and overmature trees of larger size distribute rather heterogeneously. The followings were measured in this investigation: density of yearlings and trees, height of plants, diameter breast high (over 3 cm.) or basal diameter (on the tree smaller than 3 cm. d. b. h.), diameter of crown, etc. Some standard trees also were selected from each plot for stem analysis, etc. (Tab. 3). The profile chart of the vegetation on Transect I (West) in Forest Unit III-IV is illustrated in Fig. 2, and the main results of Transect II (East) are summarized in Table 1.

Remarkable changes along with descending the slope in a forest unit are the increase of age and height, and

Table 2. Change of the densities of *Abies Veitchii* and *A. Mariesii* on Transect II (East) acrossing the middle of Forest Units III, IV and Va. The "suppressed" trees have their crowns clearly under those of "canopy trees," but they are distinguished from young growth in their morphology as well as in supposed age. The mark * shows the position of the "dead trees strip".

Forest	Situation		No. of trees per are					
Unit	m.	Plot	Canopy tree			Young growth	Sup-pressed	Dead trunk standing
		No.	<i>A. Veitchii</i>	<i>A. Mariesii</i>	Total			
III	0~5	40	28	12	40	1534	—	44
	10~15	39	32	32	64	4030	4	12
	20~25	38	20	16	36	6866	24	24
	25~30	38	8	12	20	3200	60	8
	* 30~35	37	1750	400	2150	—	—	200
	* 35~40		850	350	1200	—	—	1500
IV	40~50	36	383	508	891	—	—	353
	50~60	35	600	167	767	—	83	350
	60~70	34	125	163	288	—	25	250
	70~80	33	80	88	168	600	12	68
	80~90	32	68	72	140	4767	16	44
	90~100	31	28	20	48	6367	20	36
	100~110	30	12	16	28	7475	12	12
	110~120	29	16	36	52	—	—	52
	120~130	28	20	28	48	3460	8	36
	* 130~140	27	500	780	1280	—	—	180
V	* 140~150	26	575	450	1025	—	—	112
	150~160	25	175	137	312	—	112	200
	160~170	24	50	75	125	—	12	150
	170~180	23	48	48	96	—	20	64
	180~190	22	8	24	32	—	16	28
	190~200	21	8	12	20	—	—	12
	200~210	20	16	16	32	—	—	28
	210~220	19	12	16	28	2600	—	20
	220~230	18	4	16	20	4890	—	6
	* 230~240	17	—	2	2	1647	—	24
	* 240~250	16	—	—	—	950	—	26

trees strip, the growing trees diminished in their number rapidly towards the lower dead trees strip with a constant decreasing rate, except for in a stable state of overmature stands.

Change in age, tree diameter and height in the forest unit IV with the descending the slope will be seen in Table 3. The mean height of young trees just in the dead trees strip III was 1 m. or less, and the mature tree at 60~70 m. downwards from the strip III, reached a maximum height of 10.3 m. After Yoshida and Yamanouchi,¹²⁾

Table 3. Age, diameter breast high, and height of standard trees in Forest Unit IV. Plot Nos. correspond to those in Table 2.

Plot No.	<i>Abies Veitchii</i>			<i>Abies Mariesii</i>		
	Age	D. B. H. (cm.)	Height (m.)	Age	D. B. H. (cm.)	Height (m.)
36	17	—	0.96	18	—	1.05
35	30	2.6	2.27	29	2.2	2.05
34~33	41	4.8	3.76	41	4.4	3.70
32	61	7.4	6.40	64	7.6	6.00
30	73	10.4	7.62	78	9.2	7.55
28	86	14.0	9.60	70	14.0	10.10
27	83	14.1	10.33	97	14.6	10.25

the maximum heights of trees are different with altitude of the slope where the trees are standing, e. g. at the forest unit II (2380 m.) it was 7.6 m and at the forest unit VI (2250 m.) 13.4 m. However, comparing to the normal growth of *Abies* of 20-25 m. high on better sites, the growth of the trees on the southwest slope of Mt. Shimagare seems to be generally worse. The maximum diameter breast high of 14 cm. also seems to be obviously too small in comparison with that the mature trees have sometimes trunks of over 60 cm. d. b. h. in better sites. Mean maximal crown diameter of 5.5 m. was observed on the trees just matured standing in the plots at 30-40 m. above the dead trees strip (see Fig. 1). The overmatured trees above 10 m. upwards of the dead trees strip have rather smaller crown diameter of 2.5-3.0 m.

In general, the vigorous regeneration of the overpopulated young trees of both the *Abies* species¹²⁾, and the undersized mature and overmature trees are the characteristics in the forests on the southwest slope of Mt. Shimagare.

3. Weight of leaves, branches and trunk

On the several standard trees selected in various growing stages, the dry weight of each organs, such as leaves, branches and trunk, except for root system, were measured respectively, because the leaf amount determines the light condition on the forest floor which plays important role in the direction of plant succession, and the ratio between leaves (=photosynthetic system), and branch, trunk (and root) (=non-photosynthetic system) is an important measure for the carbohydrate economy in plants or consequently for growth of trees.^{4),5)}

Descending the slope or with increase of tree age, the dry weight of leaves on a standard tree increased, and it reached its maximum of mature tree with gradual decrease of increment rates (Fig. 4). About 2.8 kg. per plant was obtained as a maximum in *A. Mariesii*, and 1.8 kg. in *A. Veitchii*. The overmatured tree just above the dead trees strip bore a very small deteriorated crown on its top, and its leaf weight was nearly half of the maximum in the just matured tree. On the contrary the total weight of bole and branches increased steadily with age of tree (or downwards on the slope). Therefore, the ratio of non-photosynthetic system increases with age

of tree, e. g. in younger trees the ratio is only 3 (*Mariesii*)-5 (*Veitchii*), in mature ones about 5 (*Mariesii*)-10 (*Veitchii*), but in over-matured ones 19 (*Mariesii*)-24 (*Veitchii*). This suggests that the balance between photosynthesis and respiration becomes worse with overmaturing up to the death of trees.

Also it should be mentioned here that the weight of leaves in *A. Mariesii* always exceeded that of *A. Veitchii* in the same age or in the same plot number, in spite of that no difference was recognized in the weight of aerial part between two *Abies* species. Therefore, the ratio of non-photosynthetic system to photosynthetic one is larger in *A. Veitchii* than another *Abies* throughout the whole ages.

4. Light intensity in the forest

Light usually influences upon the natural regeneration of forest, as one of the most essential factors. Therefore, relative light intensity was determined with two photometers (Toshiba No. 5), a photocell of which was fixed at a considerable height over the young canopy at an opening in the dead trees strip to get the full light intensity, another was moved along the main transect I under the canopy of the forest unit IV. Those measurements were done under cloudy skies to get the values which respond to the structure of the forest canopy⁶⁾. Average value of ten observations is shown in Fig. 5.

Under the young growth at the dead trees strip III the relative light intensity was 7-25 %, but only at 10 m. downwards there came a minimal intensity of only 1-6 %, in average 4 %, under the thick vigorous growth of over populated young *Abies* trees. Hereafter the light intensity under forest canopy increased quite slowly as far as 30 m. upwards from the dead tree strip IV. The average light intensities under such a mature tree canopy were 7-11 %. In the overmatured tree zone abrupt increase of light intensity came with the deterioration of the tree crowns. At the dead trees strip IV, the value observed just upon the canopy of young growth of the

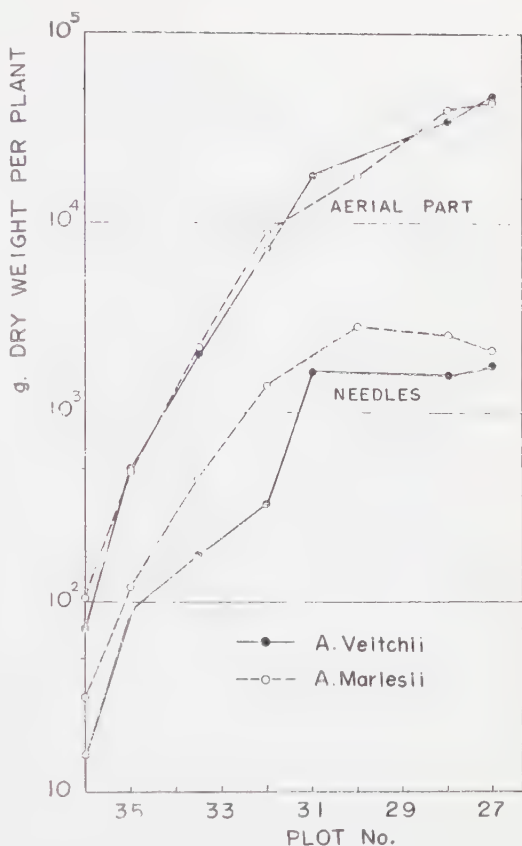


Fig. 4. Change of dry weight of needles and of total aerial part of the standard *Abies* trees in Forest Unit IV.

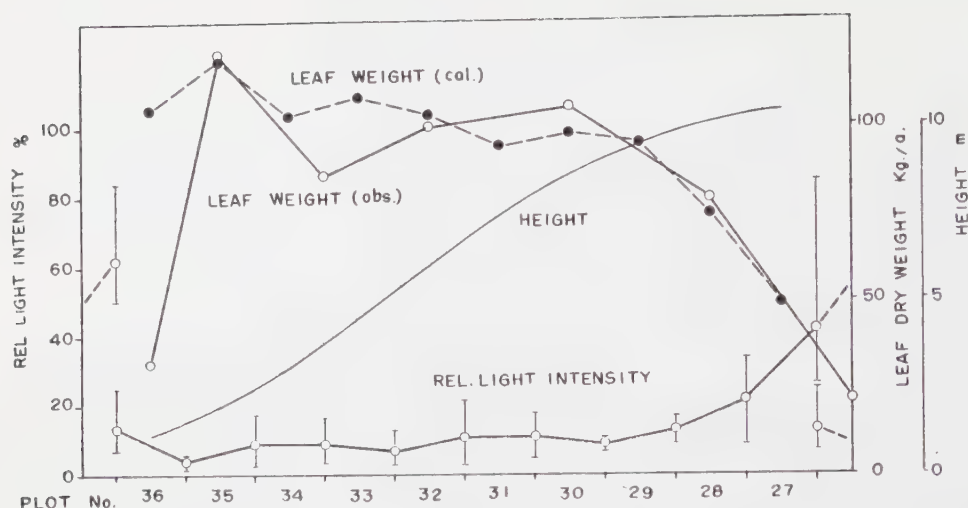


Fig. 5. Relative light intensity, height of trees and total leaf per ground area in Forest Unit IV.

forest unit V was 25–85 %, in average ca. 42 %, but under the canopy low illumination of 13 % was obtained. Young succeeding growth, or establishment of seedlings started with low density under the somewhat immature tree stands of *Abies* (Plot No. 33), in the illumination of about 10 %. The density of the tree offspring increased rapidly with the growth of the plants or spatially in a short distance downward of the slope. The highest density came under the forest stand just over-maturing. As to the tolerance of young growth, particularly as to difference between that of *A. Mariesii* and *A. Veitchii*, further investigations will be expected.

The light intensity in the plant community is mainly decided by the amount of leaves per unit ground area⁵⁾. The amount (dry weight) of leaves per are was, though very roughly, estimated from the data of leaf amount of each standard tree and the tree number per are (see Fig. 5). The value in the young growth at the dead trees strip III was 32 kg./a., but the value increased rapidly up to the maximum of 121 kg./a. at 10 m. downward from that strip. Downwards of this area somewhat constant leaf amount of 86–105 kg./a., was prevailing. From 20 m. upward from the strip IV the leaf amount decreased strikingly, and the value of overmatured stand just upward of the dead trees strip IV was only about 21 kg./a. It may be recognizable that the light intensity under canopy and the leaf amount in the canopy have just opposing trends in the variations. This will be proved more clearly by the coincidence of the leaf amount calculated from the light intensities^{4),5)} with the observed values mentioned above. The discrepancy between the two values at the youngest stage seems to be caused by interception of the vigorous growth of other herbs and shrubs in the herb stratum.

5. Undergrowth

Frequency and dominance of the undergrowth were investigated at 51 quadrats of 1 m. \times 1 m. along the transects, according to Braun-Blanquet's general estimation method. Some results are briefly summarized in Tab. 4. Almost all of the 22 species obtained in the undergrowth of this forest were the common shade plants in the subalpine coniferous forest in central Japan. The highest dominance and frequency were observed, excepting mosses, in *Pteridophyllum racemosum* which is one of the character species in *Abietum*, and Nakano^{8),9)} denominated such a sociation as *Pteridophyllosum*. However, the undergrowth species maintained in general rather poor dominance because of low illumination under the canopy of the *Abies* trees and vigorous regeneration of young growth of the dominant trees. Total standing crop of undergrowth is, generally speaking, decided by the illumination prevailing over the undergrowth^{2),6)}. Therefore, there was almost no growth in the shade of 3-4 % light intensity under the thick young growth of tree species at 10 m. downward from the dead trees strip III. With development of the forest, the obligate shade plants such as *P. racemosum* and *Oxalis japonica* arose but with low dominance. Only in

Table 4. Frequency and dominance of main species of undergrowth which included 22 species. The values were calculated from the data of 51 quadrats of 1 m. \times 1 m.

Species	Frequency (%)	Dominancy
<i>Pteridophyllum racemosum</i>	98	2~4
<i>Oxalis japonica</i>	88	1~3
<i>Carex lanceolata</i>	84	1~3
<i>Cornus canadensis</i>	73	1~2
<i>Cacalia adenostyloides</i>	63	1~2
<i>Rubus japonicus</i>	61	1~2
<i>Majanthemum bifolium</i>	59	1
<i>Pirola alpina</i>	43	+
<i>Dryopteris</i> sp.	75	1~3
Mosses	96	3~5

the place where the trees matured or overmatured and their crowns were somewhat or badly deteriorated, the vigorous growth of herbaceous or shrubby undergrowth, such as *Cacalia adenostyloides*, *Carex lanceolata*, *Cornus canadensis* was expected, accompanied by rapid development of young *Abies* trees. As to the species number in each quadrat, however, any direct relationship to the light intensities could not be recognized³⁾.

Summary

1. The subalpine coniferous forest on the southwestern slope of Mt. Shimagare in Nagano Pref., central Japan, is characterized with about six transverse "dead trees

strips" (width=10 m., length=300-800 m., interval=100 m.) where a large number of dead tree trunks of the dominant trees, *Abies Veitchii* and *A. Mariesii*, stand or fall so densely that the strips are seen as whitish stripes in a distant view.

2. The natural regeneration of dominant trees emerges under the canopy of mature trees in in-between of two dead trees strips. Vigorous young mixed growth of *Abies* species continues downwards beyond a dead trees strip of the covering canopy. Out of the canopy, the trees become larger and older, and they form a new canopy which consists of serially growing crowns, up to the overmatured and just dying trees of another dead trees strip at lower altitude.

3. Plant density, ages, heights, diameters breast high, top weights, leaf weights, undergrowth, etc., were investigated with quadrat method and in the standard trees. The density of overpopulated young trees decreased with maturing of trees by a constant rate. Vigorous regeneration of the two *Abies* species, and poor mature and overmature stands of small sized trees make the characteristics of the forest.

4. The leaf weight increased from a young tree to mature one, but in overmature tree it slightly decreased, accompanying the high increase of relative weight of non-photosynthetic system. The leaf amount in unit ground area, however, was highest at somewhat younger tree stands because of high density of young growth, and it abruptly decreased in the overmature stands.

5. The dominancy of undergrowth was markedly influenced by light intensity of the forest floor, for which the leaf weight of the dominant trees was highly responsible.

The authors wish to express their thanks to Prof. K. Hogetsu and Prof. M. Monsi for helpful suggestions and valuable advice. Their deep gratitude is also due to Mr. K. Yoshida and Mr. Y. Okanishi for kind help during the investigation. A part of the expenses of this study was defrayed with a grant-in-aid from the Research Fund of the Ministry of Education.

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摘 要

縞枯山は長野県北八ヶ岳にある標高 2395 メートルの山で、その南西斜面には約 6 条の白い縞（幅：10 メートル，長さ：300-800 メートル，間隔：100 メートル）が、主としてシラビソ，オオシラビソからなる亜高山帯針葉樹林の濃緑色の中を水平に走っている。この白い横縞はこれら針葉樹の立枯れの幹が帯状に密集してできたものである。優占樹木の芽生えはこの縞の中間にある成木の樹冠下に発生し、その下方の縞を通りぬけ、斜面を下るにしたがって樹令や大きさを増し、稚樹，成木，過熟木の段階を経て、ついには枯死木となり、一段下の縞枯部を形成している。

調査の第一歩として、斜面の中はこの一連の生育量に応じて、ワウ法，あるいは標準本法を用い、立木密度，樹令，樹高，胸高直径，地上部重，葉重，下草などを調べた。立木密度は過密な稚樹相から成木相に向かって一定の割合で減少し，成木から過熟木まではあまり減少しない。1 本あたりの葉量は稚樹から成木までは増加するが，成木から過熟木に向かっては減少し，それに伴って光合成器官（葉）の非光合成器官（幹＋枝）に対する重量比も急激に減少する。林分の単位面積あたりの葉量の最大は，縞枯部のやや下方の密生した稚樹のところにあり，それから成木のところまではあまり減少しないが，縞枯部近くで急減する。林内相対照度はこのような葉量の変化に対応した変化を示し，下草の優占度はこの林内照度によって影響されていることが明らかにされた。

Liliaceae of Dr. Hayata's Indo-Chinese Collection

by Tetsuo KOYAMA*

小山鐵夫*: 早田教授採集のインドシナ産ユリ科植物

Received June 18, 1958

This paper deals with the liliaceous specimens left unidentified by the late Dr. B. Hayata in the herbarium of the University of Tokyo (TI). His ample Indo-Chinese collections were made during his long trips to the Indo-Chinese peninsula in three times; according to his diary, he was in Tonkin from June to August in 1917, in Annam from May to June in 1921, and in Siam from September to December in 1921. He also visited Yunnan briefly in the first trip. I originally intended to identify only the sedges of the collection for a monograph of the Cyperaceae in Asia, but lately some collections from Himalayas were brought to Japan, and in the course of the determination of these plants, the need was felt to have Hayata's Indo-Chinese plants determined. The first paper comprising the taxonomic treatment of Cyperaceae of that collection was published in Contributions de l'Institut Botanique de l'Université de Montréal 70: 5-64. 1957, while the second including Xyridaceae and Eriocaulaceae appeared in the Philippine Journal of Science 84 (3): 365-378. 1956. In Hayata's collection containing mostly lowland species, the Liliaceae is not very abundant, however, there are some isotypes described by Gagnepain in Le Bulletin de la Société Botanique de France 84: 1934, since E. Lagrange, who accompanied him in his last trip, brought a part of the collection back to the Herbarium of Muséum d'Histoire Naturelle de Paris (P).

1. **Asparagus acerosus** Roxb., Fl. Ind. ed. 1, 2: 150 (1820); Baker in Journ. Linn. Soc. 14: 622 (1875); Hook. f., Fl. Brit. Ind. 6: 317 (1892); Gagnep. in H. Lecomte, Fl. Génér. Indo-China 6: 778 (1934).

Annam: Ninhua. B. Hayata. 392.—India, Malaysia, Australia.

Species well characterized by its elongate racemes 2 to 7 cm. long.

2. **Asparagus cochinchinensis** (Lour.) Merrill

Tonkin: Tamdao. B. Hayata, sin. num.—China, Korea, Japan.

3. **Asparagus filicinus** Hamilt. ex D. Don, Prodr. 49 (1825).

Annam: Dalat. B. Hayata, sin. num.—China, India.

4. **Chlorophytum orchidastrum** Lindley.

Siam: Doi Sutep. B. Hayata, sin. num.; entre Pong Pa Pow et Pong Kioh. B. Hayata, sin. num.—Africa, India, China.

5. **Dianella ensifolia** DC. ex Redouté.

* Botanical Institute, Faculty of Science, University of Tokyo 東京大学理学部植物学教室

A n n a m: Dalat. *B. Hayata*, 92.—Madagascar, India, Malaysia, Formosa, Ryukyus, Japan.

6. ***Disporum cantoniense*** (Lour.) Merrill; Kitamura in Kihara, Fauna & Fl. Nepal Himal. 92 (1955).

A n n a m: Dalat. *B. Hayata*, 196.—India, Malaysia, Formosa, Japan.

7. ***Disporum tonkinense*** T. Koyama, spec. nova a *Disporo sessili* D. Don floribus multo minoribus vix 1 cm. longis et a *Disporo cantoniensi* Merrill foliis ellipticis ovaribusve, habitu minore distincte recedit—Fig. 1.

Perennis, rhizomate lignoso breviter repente, radicibus validis 1/2–4 mm. crassis fusco-cinnamomeis. Caulis gracilis 2–4 dm. altus simplex vel sursum laxe dichotomi-ramosus basi vaginis 2–5 spathaceis membranaceisque 2–6 cm. longis fusco-rubentibus apice obtusis vestitus. Folia remote alternata ad unicum caulem 3–7 sessilia elliptica vel ovalia 2.5–6 cm. longa 1.5–3 cm. lata membranacea 3–5-nervia margine integra apice basique abrupte breviter acuta. Umbella laxa 3–5-flora sessilis vel brevissime (1–2 mm.) pedunculata, pedicellis obliquis 9–13 mm. longis.

Flores albi circiter 9 mm. longi; tepala exteriora ovato-elliptica 6–7 mm. longa trinervosa apice rotunda atque mucronata in anthesi sursum patentia usque recurva; tepala interiora exterioris similia sed leviter angustiora longioraque; stamina 6 tepalis longiora, filamentis compressis 6 mm. longis, antheris oblongis 2.5 mm. longis; pistilum cum staminibus aequilongum apice trifidum, ovario 1.5 mm. longo ellipsoideo. Bacca globosa 6–9 mm. in diametro maturitate sordide caerulea; seminibus ovoideis facie opacis 4 mm. longis ac latis.

Tonkin: Tamdao. *B. Hayata*, sin. num.—holotypus in TI.

Closest to *D. cantoniense*, but differing therefrom in having broadly elliptical, light green leaf-blades.

8. ***Disporum trabeculatum*** Gagnepain in Bull. Soc. Bot. Fr. 81: 286 (1934) et in H. Lecomte, Fl. Génér. Indo-Chine 6: 783 (1934).

Tonkin: Chapa. *B. Hayata*, sin. num.; Tamdao. *B. Hayata*, sin. num.—Endemic in Indo-China.

This species is also known from Annam; quite distinct secondary nervules transversely connecting longitudinal nerves on large broadly oval leaves clearly distinguish this one from all other members of this genus.

9. ***Gloriosa superba*** Linn.

Siam: Xen Mai. *B. Hayata*, sin. num.—Rather widely extending in the tropical



Fig. 1. *Disporum tonkinense* T. Koyama (Holotype)

regions of the Old World from northern Africa through India to Malaysia.

10. **Gonioscypha muricata** Gagnepain in Bull. Soc. Bot. Fr. **81**: 287 (1934) et in Lecomte, Fl. Génér. Indo-Chine **6**: 803, f. 79, 1-5 (1933).

Siam; Doi Stuep. *B. Hayata, sin. num.*

New to the flora of Siam, previously known only from Laos, where this was first described. The genus *Gonioscypha* is a small southern Asiatic genus including only two species of very strange look. Another species is *G. eucomoides* Baker of Himalaya, bearing dark green flowers. In our plants, flowers are whitish in the tubular part and blackish purple on segments. Gagnepain (11. cc.) described perianth tubes as 3 mm. wide, but in the above specimen, they are about 7 mm. across.

11. **Hemerocallis longituba** Miquel.

Yünnan: Yünnanfou. *B. Hayata, sin. num.*—Formosa. Japan.

This specimen well coincides with Japanese common form of *H. longituba*, which may be specifically distinct from European *H. fulva* in the longer perianth tube always 2.5-3 cm. long.

12. **Heterosmilax japonica** Kunth, Enum. Pl. **5**: 270 (1850); T. Koyama in Quart. Journ. Taiwan Mus. **10**: 20 (1957).

H. dalatensis Gagnep. loc. cit. **81**: 69 (1934), ex isotypo—*Smilax planipedunculata* Hayata—*H. arisanensis* et *raishaensis* Hayata.

Annam: Dalat. *B. Hayata, 768*—type of *H. dalatensis* Gagnep. in P, isotype in TI.—Formosa, Ryukyus.

Gagnepain described *H. dalatensis* laying stress on its oblong perianth, which is a relatively rare occurrence in the continental species of this genus, on the other hand, however, oblong connate perianth minutely 3-toothed at the apex characterizes *H. japonica* Kunth itself!

13. **Heterosmilax polyandra** Gagnepain in Bull. Soc. Bot. Fr. **81**: 70 (1934) et in H. Lecomte, Fl. Génér. Indo-Chine **6**: 775, f. 73 (1934).

Tonkin: Tamdao. *B. Hayata, sin. num.*—Endemic in Indo-China.

This plant has hitherto been known only from Laos. There is a little hesitation in identifying this specimen with *H. polyandra* from Laos, since the specimen is not quite perfect and lacks the male flower. Further in our specimen, leaf-blades are not cordate at the base but rounded.

The polyandry (9 to 12 to a flower) seen in this species characterizes the section *Pseudosmilax* (Hayata) T. Koyama, in which are described two more species, viz. *H. seisiuensis* Wang et Tang, and *H. hogoensis* Hayata, the latter being considered to be identical with the former (cf. T. Koyama, op. cit. 21. 1957).

14. **Ophiopogon latifolius** L. Rodrigues in Bull. Soc. Bot. Fr. **75**: 998 (1929)—Fig. 2.

Tonkin: Tamdao. *B. Hayata, sin. num.*—Endemic in Tonkin.

Very stiff broad leaves gradually narrowed below to a petiole-like base well define this strange looking species, which seems to be near to *O. dracaenoides* Baker of Himalaya. Besides this one, two new species, *O. subverticillatus* Gagnep ex

L. Rodr., and *O. tonkinensis* L. Rodr., were published in the same place, however, none of these was included in Flore générale de l'Indo-Chine tome 6 by Gagnepain (1934).

15. ***Polygonatum laoticum*** Gagnepain in Bull. Soc. Bot. Fr. 81: 288 (1934) et in H. Lecomte, Fl. Génér. Indo-Chine 6: 794 (1934).

Stem robust, simple, 3-6 dm. tall, obtuse-angled; leaves 7 to 13 to a stem, alternate, elliptical or ovate-oval, membranous, suddenly contracted to sessile or shortly petiolate (up to 5 mm. lg.) base apex abruptly attenuate and short-caudate; flowers in groups of 2 to 3 disposing in sessile axillary umbel; berries 6 to 12 mm. across, blue-black seeds 1 to 5 in a berry.

Annam: without definite locality. *B. Hayata, sin. num.*

As this specimen lacks the flower, I am not quite sure that this plant is quite correctly identified.

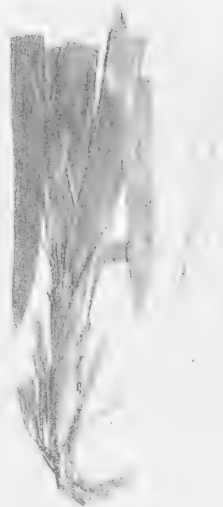


Fig. 2. *Ophiopogon latifolium* L. Rodr.



Fig. 3. *Smilax brevipes* T. Koyama (Holotype.)

16. ***Smilax brevipes*** T. Koyama, spec. nova ex affinitate *Smilacis siderophyllae* H. Mazz. differt a qua ramis non costatis, umbellis semper simplicibus unicis petiolo multo brevioribus, et a *Smilaci cambodiana* Gagnep. recedit pedunculis umbellarum basi non squamatis, ramis ramulisque laevissimis. Sect. *Coilanthus* DC.—Fig. 3.

Liana sempervirens, caulibus graciliter elongatis teretibus laevissimis. Folia remote alternata; laminae oblongo-ellipticae usque ovaes 5-15 cm. longae 2-9 cm. latae coriaceae supra nitidae subtus opacae sed non cinereae basi abrupte contractae rotundae apice etiam rotundae cum acumine abrupte acuto, nervis principalibus longitudinalibus 3 convexis nervulis lateralibus pertenuibus obliquis reticulationes minutas formantibus; petioli 10-15 mm. longi laeves infra medium alati; claviculae breves cum petiolo suo aequilongae. Umbellae solitariae simplices subsessiles dense plus quam 30-florae, pedunculo tereti 3-7 mm. longo ebracteato 1/3-1/4 petiolum aequilongo, pedicelis capi-

llaribus ad 25 mm longis ascendentibus. Flores masculi parvuli 2 mm. longi 3 mm. lati in anthesi; tepala exteriora ovata naviculares apice rotunda; tepala interiora exterioris similia sed aliquantulum angustiora; stamina 6, filamentis multo brevioribus quam anthera ovoideo-globosa. Flos foemineus ignotus.

Siam: entre Iaksê et Bannhikh. *B. Hayata*, sin. num.—holotypus in TI.

From the external appearances, this greenbrier is most closely allied to *S. siderophylla* H.-Mazz. of Yünnan, from which it is distinguished by its always solitary umbels on peduncle not exceeding its petiole. The slender quite smooth stem and the nature of umbel suggest that this may be near to *S. glabra* Roxb., a well known narrow-leaved one.

17. **Smilax chapaensis** Gagnepain in Bull. Soc. Bot. Fr. **81**: 71 (1934) et in H. Lecomte, Fl. Génér. Indo-Chine **6**: 766 (1934).

Tonkin: Chapa. *B. Hayata*, sin. num.—Endemic in Tonkin.

Large liane with densely aculeate, coarse, woody stem.

18. **Smilax China** Linn. var. **taiheiensis** (Hayata) T. Koyama in Quart. Journ. Taiwan Museum **10**: 9 (1957).

S. verticalis Gagnepain, loc. cit. **81**: 74 (1934).

Annam: Ba Me Thout. *B. Hayata*, sin. num., allotype of *S. verticalis*!—Siam: Pong Kioh. *B. Hayata*, sin. num. entre Pong Pa Pow et Pong Kioh. *B. Hayata*, sin. num.—Formosa, S. China.

18. **Smilax ferox** Wallich ex A. DC.

Annam: Krong Pha. *B. Hayata*, 784, 979, & 981—India, S. China.

19. **Smilax glaucophylla** Klotzsch in Reise Prinz. Wald. Bot. 45, t. 91 (1862); Kitamura in Kihara, Fauna & Fl. Nepal Himal. **96** (1955); T. Koyama in Quart. Journ. Taiwan Mus. **10**: (1957).—*S. parvifolia* Wallich ex Hook f.

Yünnan: Yünnanfou. *B. Hayata*, sin. num.—India, Formosa (var.).

This plant resembles *S. vaginata* Decne. at a glance, from which it is distinguished in its somewhat climbing stem and distinctly cirrhone petioles.

20. **Smilax hypoglauca** Benthams, Fl. Hongk. 369 (1861); A. DC., Monogr. **1**: 61 (1878); C. H. Wright in Journ. Linn. Soc. **36**: 98 (1903); Norton in Sargent, Pl. Wils. **3**: 10 (1916).

Siam; Doi Suteh. *B. Hayata*, sin. num.—China (Yünnan, Hongkong, Kwantung, &c.).

New to the flora of Siam, different from its nearest alliance, *S. glabra* Roxb., in having obtuse-tipped leaf-blades.

21. **Smilax lanceaefolia** Roxburgh (Hort. Beng. 72. 1814, nomen) Fl. Ind. **3**: 792 (1832); A. DC., Monogr. **1**: 57 (1878); Hook. f., Fl. Brit. India **6**: 308 (1892); Norton in Sargent, Pl. Wilson. **3**: 11 (1916); Gagnep. in H. Lecomte, loc. cit. **6**: 767 (1934).

Siam: entre Huoi Hai and Nam Tao. *B. Hayata*, sin. num.; Doi Suteh. *B. Hayata*, sin. num.—India, China.

This large liane with large herbaceous ovate to lanceolate-ovate leaves is, I think,

a member of the *S. glabra* group, which is defined by slender, smooth, terete stem and relatively short peduncle without any scale at the base.

22. ***Smilax macrophylla*** Roxburgh (Hort. Beng. 72. 1814, nomen) Fl. Ind. 3: 793 (1832); A. DC., Monogr. 1: 193 (1878), excl. var. β .

S. ovalifolia Roxb., loc. cit. 794; Wight, Icon. 3: t. 809 (1844)—*S. retusa* Roxb.—*S. Roxburghii* Kunth.

Siam: Doi Sutep. *B. Hayata*, sin. num.—India.

23. ***Smilax opaca*** (A. DC.) Norton in Sargent, Pl. Wilson. 3: 11 (1916); Gagnep. in H. Lecomte, loc. cit. 6: 767 (1934); T. Koyama in Quart. Journ. Taiwan Mus. 10: 19 (1957).

S. lanceaefolia Roxb. var. *opaca* A. DC., Monogr. 1: 57 (1878)—*S. lanceaefolia* sensu auct. plur. non Roxb.

Siam: Nakay. *B. Hayata*, sin. num.—China, Formosa.

Many authors have confused this with *S. lanceaefolia* Roxb. It is true that the two strikingly akin when we examine sterile branches, but, although the leaves of *S. lanceaefolia* are considerably variable, they are rather thin membraneous with minute red-brown spots on the under surface, and shortly caudate at the apex, while in *S. opaca*, leaves are slightly smaller, somewhat thick membraneous, and relatively suddenly attenuate at the tip. As I mentioned in Quart. Journ. Taiwan Mus. 10: 3-4, quite different state of inflorescences suggests us that these two are better regarded as a member of different section respectively, i. e. *S. lanceaefolia* is placed in the section *Coilanthus* including *S. China* *S. glabra* etc., and *S. opaca* belongs to the section *Smilax* with clearly prophyllate peduncle of umbel, including *S. perfoliata*, *S. macrophylla*, etc.

Some plants of *S. opaca* from Siam have armed stem, and they are newly described below:

forma ***aculeata*** T. Koyama, f. nova caule aculeato.

Siam: Doi Sutep. *B. Hayata*, sin. num., type in TI—Banhikh à Taktooug. *B. Hayata*, sin. num.

24. ***Smilax perfoliata*** Loureiro, Fl. Cochinch. 622 (1790); Gagnepain in H. Lecomte, Fl. Génér. Indo-Chine 6: 761 (1934); T. Koyama in Quart. Journ. Taiwan Mus. 10: 16 (1957).

Tonkin: Tamdao. *B. Hayata*, sin. num.—Siam: Doi Sutep. *B. Hayata*, sin. num.; entre Bau Do Mo et Khou Vai. *B. Hayata*, sin. num.—S. China, Formosa, Philippines.

This plant is often confused with Indian *S. prolifera* Roxb having compound inflorescence. The auricles at the base of petiole is broader in *S. perfoliata* than in *S. prolifera*.

25. ***Smilax bracteata*** Presl, Rel. Haenk. 1: 131 (1827); T. Koyama in Quart. Journ Taiwan Mus. 10: 18 (1957).

S. stenopetala A. Gray, Bot. of Japan 412 (1859); Gagnep. in H. Lecomte, loc. cit.

6: 763 (1934).

var. **Klotzschii** (Kunth) T. Koyama in Quart. Journ. Taiwan Mus. **10**: 20 (1957) in adnota—*S. Klotzschii* Kunth, Enum. Pl. **5**: 245 (1850); A. DC., Monogr. **1**: 188 (1878).

Tonkin: Chapa. B. Hayata, sin. num.—Malaysia. New. for Indo-China.

var. **timorensis** (Blume ex A. DC.) T. Koyama in Quart. Journ. Taiwan Mus. **10**: 20 (1957) in adnota—*S. timorensis* Blume ex A. DC., Monogr. **1**: 189 (1878).

Siam: Doi Sutep. B. Hayata, sin. num.—Malaysia.

This is also new to Indo-China. In my previous paper, four eastern Asiatic greenbriers, *S. bracteata*, *S. verruculosa*, *S. timorensis*, and *S. Klotzschii*, are united into one species, of which the correct name is *S. bracteata*. *S. bracteata* originally reported from Luzon, is the same plant with one which we have called *S. stenopetala*. This species is distributed from the southern Japan through the Ryukyus and Formosa southward to Philippines, but is never reported from the Asiatic continent. *S. timorensis* is, on the one hand, extending from Indo-China southeastward to Timor Island, where the typical *S. bracteata* does not occur. But, the morphological difference between these two is very slight. *S. timorensis* is separated from *S. bracteata* only by more robust habit and somewhat caudate ovate leaves only. It is, therefore, adequate to treat *S. timorensis* as a geographical variant of *S. bracteata*. Further, an interesting matter is that each of these two has its variations, one having verruculose stem and another with smooth stem within their distributional area respectively. *S. verruculosa* is attributable to *S. bracteata*, (cf. T. Koyama, l. c.), while *S. Klotzschii* corresponds to *S. timorensis*.

ADDENDA

Mr. Raymond was kind enough to write to me saying that I should correct the following name of *Carex*, which I have named after its type locality Mt. Sutep in Siam.

Carex (Filicinae) doisutepensis T. Koyama in Le Nat. Canad. **82**: (10-11); 196 (1955), errore *doistepensis*.

摘 要

早田教授は1917年から1921年にわたり、インドシナで植物を採集されたが、標本は未同定で東大腊葉庫に蔵されている。筆者は一昨年単子葉植物の同定をしてすでにカヤツリグサ科・ホシクサ科・トウエンソウ科等を発表してきたがここに登載した一篇はユリ科を扱つたものである。新種 *Disporum tonkinense* は葉の形が丸い点ではっきりしたものであり、サルトリバイラ属の標本に *Smilax lanceaefolia* のたいへんよい標本があって、類似品 *S. opaca* との区別を肯定しえたことは収穫であつたと思う。これらの同定を通じてじょじょに台湾とインドシナのフロラの関係が明らかになること、さらに最近ようやく解明されつつあるマレーシアのフロラとの関連等、興味深いものがある。

Observational and Experimental Studies of Sensitive Plants.

X. On the Fixation of Threadlike Apparatus in Cortex of Petiole*

by Hideo TORIYAMA**

鳥山英三*: オシロイソウの研究 X. 葉柄皮層における紐状装置の固定について

Received June 9, 1958

The author recently reported that in the petiole of *Mimosa pudica* L. there are threadlike apparatus which have a connection with tannin vacuoles (Toriyama 1955). These threadlike apparatus have been hitherto overlooked or ignored by plant anatomists or physiologists, both in the study on living and fixed materials. During the past three years, the author has made an effort to get data which are of immediate relevance to the case of this threadlike apparatus. In the previous paper, the data concerning vital staining of the threadlike apparatus was described (Toriyama 1957). The purpose of the present work was to obtain a more complete knowledge of the nature of the threadlike apparatus by means of fixing methods together with some experiments.

This study has made, using materials similar to those, reported in paper VII (1957 *l. c.*). In order to observe the parenchyma in the petiole before the bending movement, the plants were as usual exposed to ether vapour for 20 minutes, thus rendering the petiole of these plants incapable of responding to any stimuli. The material in the present investigation consisted chiefly of those plants which had not received any stimuli. The fixing methods were made on the petiole to examine their cytological reactions. The details shall be described in the following paragraphs.

Observations and Experiments

a) *Results through classical cytological methods.* Results obtained by classical cytological methods are briefly summarized in the remarks of table 1. The detailed explanations concerning these results are described as follows under the topics of fixatives employed.

(1) *Kaiser's fluid.**** After having fixed for 5 hours in this fluid, the material was directly removed to 70 per cent alcohol, added a few drops of iodine solution, and then to absolute alcohol, butyl alcohol, butyl alcohol paraffin successively, and finally embedded in paraffin as usual. Paraffin blocks were cut 10 micra in thickness. The

* Contribution No. 28, from Biological Section, Tokyo Woman's Christian College.

** Biological Section, Tokyo Woman's Christian College. 東京女子大学生物学教室

*** Kaiser's fluid is composed of 10 g. of sublimate, glacial acetic acid 3 ml. and distilled water 300 ml.,

Table 1

Fixatives	Result of fixation	Remarks
Kaiser's fluid	+	slender
Susa's fluid	+	slender rosary-like
Bouin's fluid	+ +	slender rosary-like
Neutral formalin	+	slender
Müller's fluid	+	rosary-like
Carnoy's fluid	—	
Bensley's fluid	+ +	slender
Champy's fluid	+ +	slender
Altmann's fluid	+ +	slender

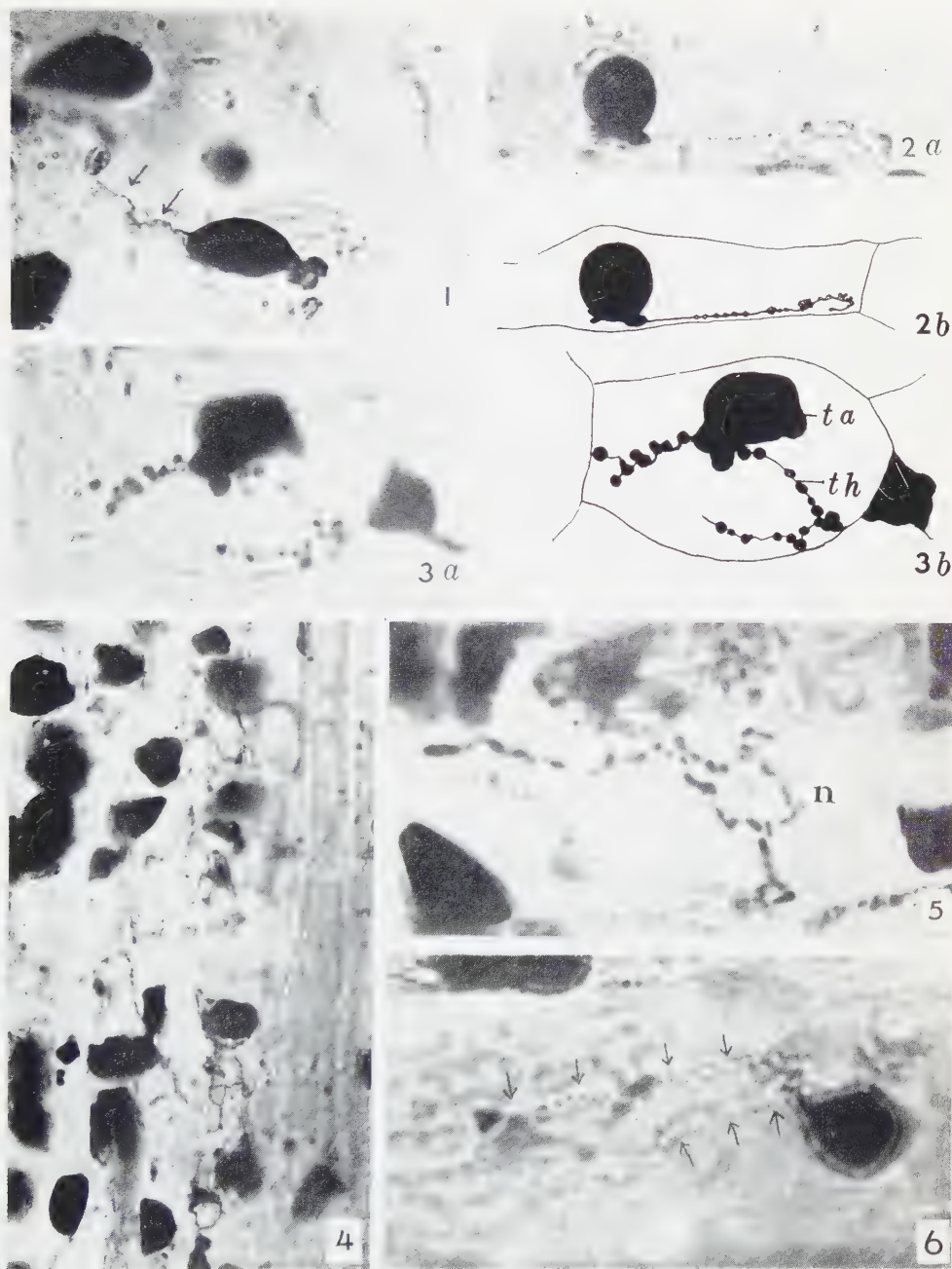
sections were stained with 0.5 per cent alcoholic toluidine blue solution which was very convenient for differentiating the threadlike apparatus and the tannin vacuole. Kaiser-toluidine blue preparation showed a good result (Fig. 1). The thread and tannin vacuoles stain greenish blue by this dye solution, assuming a similar status to the apparatus moderately stained with vital staining (Toriyama 1957 *l. c.*). The cell wall, cytoplasm, nucleus, chloroplast and other cellular elements are not however stained with this solution.

(2) *Susa's fluid*.* The proper duration of the fixation was about 24 hours. Fixed material was then washed out with 95 per cent alcohol. The dehydration was performed through a series of tertiary butyl alcohol, and was finally embedded in paraffin as usual. Paraffin blocks were cut 10 micra in thickness. The sections were stained with 0.5 per cent alcoholic toluidine blue solution. The threadlike apparatus in a rosary-like form stains very intensely blue. The toluidine blue staining revealed good results concerning the thread and tannin vacuole. But the only regrettable fact was that the cell shrink a little (Figs. 2a, b and 3a, b).

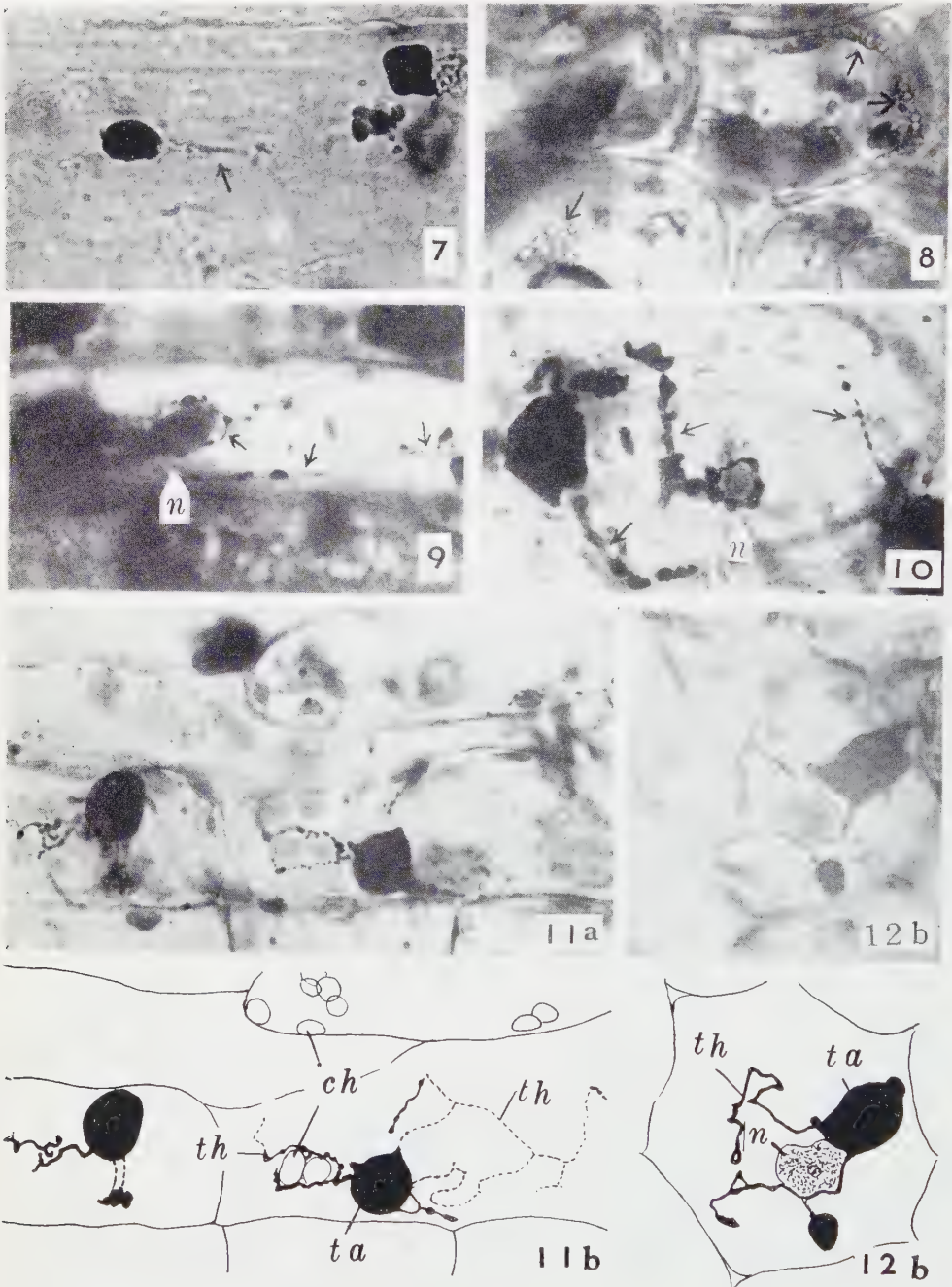
(3) *Bouin's fluid*** The moderate duration of fixation was about 4 hours usually. After fixation the material was washed in running water overnight. The dehydration and cutting procedure were performed in the same manner as in the foregoing method. Then, the sections were stained with alcoholic toluidine blue solution. By this technique, the threadlike structure appeared very clearly (Figs. 4, 5, 6). Sometimes, the threadlike apparatus appeared a little broader than those in the other preparation (Figs. 5, 6).

* Susa's fluid is composed of 50 g. of mercuric chloride, 2 g. of trichloroacetic acid, 20 ml. of formalin, 4 ml. of glacial acetic acid and 30 ml. distilled water.

** Bouin's fluid is composed of 75 ml. of picric acid, 25 ml. of formalin and 5 ml. of glacial acetic acid.



Figs. 1-6. Threadlike apparatus in the lacunal parenchymatous cell of petiole. $\times 800$. 1-fixed with Kaiser's fluid; 2a, b and 3a, b-fixed with Susa's fluid; 4($\times 320$) 5, 6-fixed with Bouin's fluid; All materials are stained 0.5% alcoholic toluidine blue. ta-tannin vacuole; th-threadlike apparatus; n nucleus.



Figs. 7-12. Threadlike apparatus in the parenchymatous cell of petiole. $\times 800$. 7-fixed with 10% neutral formalin; 8-fixed with Müller's fluid. Both materials are stained 0.5% alcoholic toluidine blue. 9, 10-fixed with Bensley's fluid; 11a, b-fixed with Champy's fluid; 12a, b-fixed with Altmann's fluid. Arrows indicated the threadlike apparatus. n-nucleus; th-threadlike apparatus; ta-tannin vacuole; ch.-chloroplast.

(4) *Neutral formalin.* The commercial formalin neutralized with calcium carbonate was diluted with distilled water in proportion of 1:5. The proper duration of fixation by this fixative was 24 hours. The washing and dehydration procedure are performed in the same manner as in the foregoing methods. The material was then cut 10 to 15 micra in thickness. The sections were stained 0.5 per cent toluidine blue solution. The adequate duration of fixation in the dye solution was determined only from experience. For the present material 10 hours staining in the dye solution was quite enough to stain the threadlike structure and the tannin vacuole. It must be noted that the thread assumes a slender feature by the fixation of neutral formalin (Fig. 7).

(5) *Müller's fluid.** The adequate duration of fixation was about 10 hours. After fixation the material was washed in running water overnight. The dehydration and cutting procedure were performed in the same manner as in the foregoing methods. The sections were stained with alcoholic toluidine blue as usual. The thread and tannin vacuole were also found to be well preserved by Müller's fluid. The thread and the tannin vacuole were equally stained in a greenish blue color. It is remarkable that the thread takes a rosary-like feature as shown in Fig. 8.

(6) *Carnoy's fluid.*** The material was fixed in this fluid for 5 hours. After completion of the fixation the material was dehydrated in absolute alcohol. The cutting and staining procedure were performed as usual. By this technique, the cell wall and the cytoplasm stained light blue. After this fixing procedure the threadlike apparatus and tannin vacuole were found to be mingled with each other, both being stained light blue color. Consequently, the fixing figure of the thread and tannin vacuole were not observable.

The available data from the experiments concerning the fixing of threadlike apparatus may be summarized as follows. Among various fixatives, such as Kaiser's fluid, Susa's fluid, Bouin's fluid, Müller's fluid and simple neutral formalin used, the best results were obtained by using Bouin's fluid.

b) *Results through new methods.* Other than the above mentioned methods, the author attempted the following methods using osmium tetroxide. With a purpose to know the fine structure of the thread, the author tried new methods upon the petiole. First he employed Bensley's fluid.*** Small pieces of tissue were kept in the fixing fluid, the adequate duration of fixation being about 10 hours. After completion of fixation they were thoroughly washed in running water for 10 hours to remove any traces of the reagents. These materials were cut 20 to 40 micra in thickness by means of a hand microtome. The sections were washed in distilled water, which was

* Müller's fluid is composed of 2.5 g. of potassium bichromate, 1 g. of sodium sulphate and distilled water 100 ml..

** Carnoy's fluid is composed of 60 ml. of absolute alcohol, 30 ml. of chloroform and 10 ml. of glacial acetic acid.

*** Bensley's fluid is composed of 4 ml. of 2.5 % aqueous sublimate, 1 ml. of 2 % osmium.

changed for several times. The sections were mounted in pure glycerin, without any subsequent staining. This technique gives not only excellent result in demonstrating the thread, but also is outstanding in the fixation of nuclei. By dint of this technique, the threads were clearly observable even before reception of stimuli in the parenchymatous cell. The threads in this case have a tendency to form a cluster around the nuclei as shown in figures 9 and 10. After receiving a stimulus, the same figure was still observable in the cell. In addition, these threadlike apparatus were also found in the parenchyma of the transitional portion from the petiole to primary pulvinus. Figure 10 shows the apparatus of this portion, the material being fixed with Bensley's fluid. As the thread of these materials was very favorable for detailed study, the author used this technique for the observation of various features of the threads.

Next, the material was fixed with Champy's fluid.* The adequate fixation with this fixative was only known from experience. For the present materials, 20 hours-fixing in Champy's fluid was quite enough to fix the thread in the cell. After fixation the material was thoroughly washed in running water for 10 hours to remove any trace of the fixing reagents. These materials were then cut 20 to 40 micra in thickness. The sections were also mounted in pure glycerin. By this fixing fluid, the thread does not assume the rosary-like form (Figs. 11a, b). The thread and tannin vacuole are tinged in black color by osmic acid, while the nuclei are colored light brown. The thread assumes various features. Sometimes they develop to the extent of assuming a long slender threadlike apparatus. Before receiving the stimulus, the cytoplasm and the threadlike apparatus were not mixed with each other, as in the case of Carnoy's fluid. After being stimulated the same figure was still observable in the cell.

Thirdly, the material was fixed with Altmann's fluid.** The fixing and washing procedure were performed in the same manner as before. By this technique, the difference in the parenchymatous cell before and after the bending movement could not be observed. The threads appear uniformly in the form of slender threads. As an effect of the fixation, the threads have a tendency of gathering themselves in a cluster around the nuclei as shown in Figures 12a, b. These threads are blackened by the osmium tetroxide. The cytoplasm is not markedly differentiated from the cell wall. Both the threads and the tannin vacuoles were stained black within 10 hours, showing no differences color. The available data from the above mentioned results may be summarized as follows. The threadlike apparatus stains black by osmium tetroxide, and takes a slender form.

The available data on the fixation concerning the threadlike apparatus in the lacunal parenchyma must be mentioned as follows. Contrary to my expectation, the

* Champy's fluid is composed of 35 ml. of 3 % aqueous solution of potassium bichromate, 35 ml. of 1 % chromic acid solution and 20 ml. of 2 % osmium.

** Altmann's fluid is composed of 10 ml. of 5 % aqueous potassium bichromate and 10 ml. of aqueous 2 % osmic acid.

threads do not join one cell with another cell lengthwise, nor do they connect with the lateral neighbouring cells.

Discussion

Many attempts have been made in order to make clear the morphology of the canaliculi or vacuome in the plant cells. Among these attempts the successful observations of the canaliculi have been obtained upon the meristem cells (Holmgren 1899, Guilliermond 1922, Gicklhorn 1926 *et. al*). During course of the study reported in the fourth paper of the present series of investigations, the author found a threadlike structure in the lacunal parenchyma of *Mimosa* petiole. Recently, the author has reported that basic dyes stain the thread in the cortex of the petiole before and after receiving the stimulus. It was confirmed that the threads possess a lipoidal nature from the staining properties with vital dyes. Furthermore, the author postulates that the thread is one of cellular elements, and is not mere artificial figure (Toriyama, 1957 *l. c*).

In order to know the further detailed cytological and chemical natures of the apparatus, the author attempted to use some fixing techniques. It seems important for this purpose that the fixatives adopted should list the form of the apparatus as modified artificially or solved by the reagents. Various fixatives, such as Kaiser's fluid, Susa's fluid, Bouin's fluid, Müller's fluid, Carnoy's fluid, or simply 10 per cent neutral formalin were used. Among them the best results were obtained with the combination of Bouin's-toluidine blue. By using alcoholic toluidine blue, the tannin vacuole and threadlike apparatus were stained very strongly and the contour of the thread had a clear-cut appearance. By means of the author's new technique, the analysis of their finer architecture became easy. In other words, the fixatives containing bichromate and osmic acid have proved to be excellent for this purpose. Osmic acid on the other hand stains thread positively. This reaction probably shows that the accumulation of tannin substance occurs in the thread. It would not be too much to say that the functional significance of a threadlike apparatus is the accumulation of the tannin substance. Therefore, these two morphologically different kinds of elements should be placed in the same category. In other words, the threadlike apparatus may be called a threadlike vacuome, which reminds us of the filamentous vacuole as reported by Bailey (1930). Zirkle (1932) also observed the canal and net-like vacuole in *Pinus Strobus*. These vacuoles and that of the *Mimosa* may be placed in different categories.

The author found that the threads assume rosary-shapes or slender threads in the living condition as shown by the vital staining. These threads are, on the other hand, capable of bending both under the natural as well as under the artificial condition (Toriyama 1957 *l. c.*). In the fixed material, their shapes show many modifications, and often shows to be curious. In some cells *e. g.* those of the parenchyma of the transitional tissue, the threadlike apparatus usually appear attached to the

nucleus. Sometimes chloroplasts draw near to the threadlike apparatus, showing a close relationship between the two (Figs. 11a, b). These phenomena seem to be very suggestive.

The main obstacle to the chemical investigation has been so far the difficulty of obtaining a pure sample of the threadlike apparatus and tannin vacuole. The detailed chemical nature and the distribution of these threads are expected to be the subjects of future research. Furthermore, the questions of the structure and function of the threadlike apparatus remain unsolved for future studies.

Summary

In the present investigation, the modes of the fixation of the threadlike apparatus in the cell of *Mimosa* petiole were studied from the cytological view-point. The experimental results obtained are summarized below.

1) Threadlike apparatus and tannin vacuole in the lacunal parenchymatous cell of the petiole are chiefly dealt with.

2) Threadlike apparatus and tannin vacuole were fixed with Kaiser's fluid, Susa's fluid, Bouin's fluid, Müller's fluid and neutral formalin. They were stained with a 0.5 per cent alcoholic solution of toluidine blue. The combination of Bouin's fluid and toluidine blue was found to be a favorable and a specific method for staining the thread and tannin vacuole.

3) By Carnoy's fluid, the thread and tannin vacuole are inadequately fixed, and are dissolved and mixed with each other.

4) By fixing the lacunal parenchyma of the petiole with the fixatives containing osmium tetroxide, *i. e.* with Bensley's, Champy's and Altmann's fluid, the thread and tannin vacuole are observed in black staining.

5) Judging from the data obtained by these experiments, it is concluded that the thread is one of the cellular elements, and is by no means a mere artificial figure.

6) The threadlike apparatus do not join one cell with another cell lengthwise, nor connect the lateral neighbouring cells.

Acknowledgment

The present author wishes to express hearty thanks to Prof. Sirô Tarao who kindly offered criticism concerning this work.

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摘 要

オジギソウ (*Mimosa pudica* L.) の葉柄の皮層、とくに細胞間隙が大きく発達している部分の柔細胞中にはタンニン液胞と紐状装置が認められるが、これらは塩基性色素で生体染色することができる (Toriyama 1955, 1957)。この紐状装置は次に述べる方法によっても明瞭に観察することができる。Kaiser 液、Susa 液、Bouin 液、Müller 液、およびホルマリンで固定した後パラフィン切片とし、これらをそれぞれトリジンブルーのアルコール溶液 (0.5%) で染色する。この方法によると細胞膜や細胞質は染色されず、紐状装置とタンニン液胞が青色に染色され、Carnoy 液でこれら紐状装置とタンニン液胞を固定されない。また筆者創案の方法、すなわち、Bensley 液、Champy 液、Altmann 液等で固定した材料を水洗した後、ハンドミキサーで碎き、中出しし、染色を行なう。この場合グリセリンに封じて検鏡する。この方法は細胞膜および細胞質は染色されていないので、細胞内の観察に好都合である。紐状装置およびタンニン液胞の色素成分は、この染色固定法により非常に明瞭に認められ、観察することができる。以上の観察による紐状装置の構造は、これまで知られていなかったの細胞要素と考えられる。

Short Communication

Über den lichtunempfindlichen Teilprozess in der blühinduzierenden Dunkelperiode bei *Pharbitis Nil* (Vorläufige Mitteilung)

von Atsushi TAKIMOTO,* Katsuhiko IKEDA* und Shun-ichiro IMAMURA*

瀧本 敦* 池田勝彦* 今村駿一郎*: アサガオの花成の促進される暗期における比較的光に安定な過程について (予報)

Eingegangen am 15. August 1958

Es ist bekannt, dass bei vielen Kurztagpflanzen die auf schwaches Licht von genügender Dauer folgende Dunkelperiode auf die Blütenbildung unwirksam ist.^{1),3)} Bei *Pharbitis Nil* trifft dies ebenfalls zu. Wenn aber die Dauer der Vorbeleuchtung mit schwachem Licht abgekürzt ist, nämlich bis auf 4 Stunden, kommen die Pflanzen, selbst nach einer Dunkelperiode von subkritischer Dauer, zur Ausbildung der Blütenanlagen. Der Versuch wurde mit Keimlingen von *Pharbitis Nil*, Sorte, „Violett“, ausgeführt, die durch eine einzige induktive Dunkelperiode zur Blütenbildung gebracht werden können²⁾. Die kritische Dunkelperiode unter den günstigsten Bedingungen ist etwa 9 Stunden.

Die Samen wurden mit konzentrierter Schwefelsäure 20 Minuten lang behandelt, gründlich gewaschen und darauf im Leitungswasser zur Quellung gebracht. Nach 2 Tagen wurden die gleichmässig keimenden Samen in Töpfen ausgesät, und im Gewächshaus bei $30 \pm 2^\circ$, unter Dauerlicht (natürliches Tageslicht und nächtliche Beleuchtung

* Laboratorium für Angewandte Botanik, Landwirtschaftliche Fakultät, Kyoto Universität
京都大学農学部応用植物学研究室

mit etwa 500 Lux von Glühlampen), aufgezogen. Die Versuche sind 3 Tage nach der Aussaat ausgeführt worden und nach der experimentellen Behandlung wurden die Pflanzen unter kontinuierlicher Beleuchtung gezogen. Etwa zwei Wochen nachher wurden sie zur Beobachtung gebracht.

Vor der Dunkelbehandlung von 12, 10, 9, 8 und 6 Stunden bei $25 \pm 1^\circ$ waren die Versuchspflanzen 4 Stunden lang schwachem Licht fluoreszierender Lampen ausgesetzt (ca. 10 Lux), und die Kontrollpflanzen wurden ebenfalls 4 Stunden lang unter starkem Licht (ca. 4000 Lux) gehalten. Andere Gruppen von Kontrollpflanzen wurden von Anfang der Vorbeleuchtung der Versuchspflanzen an 16, 14, 12 und 10 Stunden lang im Dunkeln gehalten.

Die Resultate sind in Tabelle 1 dargestellt. Die auf Starklicht folgende Dunkelperiode von 8 bis 10 Stunden löste keine Blütenbildung aus. Wurden die Pflanzen aber vor der 8- bis 10-stündigen Dunkelperiode 4 Stunden lang im Licht schwacher

Tabelle 1. Einfluss des 4-stündigen schwachen Lichtes auf die Blühreaktion in der darauf folgenden Dunkelperiode.

Vorbestrahlung	Dauer der Dunkelperiode in Std.	Zahl der Versuchspflanzen	Blühprozent
4-stündiges Schwachlicht von 10 Lux	12	37	100
	10	40	100
	9	37	100
	8	40	52,5
	6	40	0
4-stündiges Starklicht von 4000 Lux	12	39	15,4
	10	36	0
	9	40	0
	8	39	0
	6	40	0
Sonnenlicht	16*	39	100
	14*	40	100
	12*	40	60,0
	10*	39	0
16-stündiges Schwachlicht		40	0

* Die Pflanzen wurden am Beginn des Versuchs in eine Dunkelkammer versetzt.

Intensität gehalten, so war die Blütenbildung erheblich gefördert, d. h. die Pflanzen bildeten eben so viel Blüten wie die Kontrollpflanzen, welche von Anfang an 12 bis 14 Stunden lang im Dunkeln gehalten wurden. Die Pflanzen, welche im schwachen Licht 16 Stunden lang gehalten wurden, bildeten keine Blütenanlagen.

Die Anfangsphase der blühinduzierenden Dunkelperiode erfordert also nicht immer vollkommene Dunkelheit.

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プログラム

第23回

福岡
1958

日本植物学会大会

学会会長 服部 静 夫

大会名誉会長 瀬 瀬 理 一 郎

大会会長 小 島 均

会 期 昭和33年10月25日(土)—27日(月)

会 場 九州大学農学部・理学部(福岡市箱崎)

日 程

日	時 会場	午前				昼	午後				夜			
		9.00	10.00	11.00	12.00		1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00
25 (土)	A	受 付	生 理 A1—12			展 示 講 演 (1・2)	生 理 A13—26			植物分類学会 形態部会				
	B		生 理・生 化 B1—12				生 理・生 化 B13—27							
	C		生 態 C1—11				細 胞 C12—26							
	D		形態・細胞・分類 D1—11				形 態・分 類・地 理 D12—26							
26 (日)	A	シンポジウム 3 (生 理)			展 示 講 演 (3・4)	生 理 A27—41			生態部会 藻類学会 生理談話会					
	B	シンポジウム 1 (分 類)				生 理 B28—42								
	C	シンポジウム 2 (細 胞)				形態・細胞・分類 C27—44								
	D					生 態 D27—41								
27 (月)	A	シンポジウム 6 (形 態)				細胞・遺伝 A42—48			記 念 撮 影	総 会	懇 親 会			
	B	シンポジウム 4 (生 態)				生 理 B43—49								
	C	シンポジウム 5 (遺 伝)				生 理 C45—51								
	D					形 態 D42—48								
28 (火)	(市内見学および旅行出発)													

展 示 講 演 (講演者の都合がつけば、日程中に示した時間のほかにも講演していただく予定です)

25 日

- | | | |
|-------------------|------------------------|------|
| (1) 岡田喜一 (長崎大・水産) | ら節竹 (一名らせん竹) | F 会場 |
| (2) 藤原 勲 (佐賀大・文理) | オオバコ属における倍数種の合成—六倍種の合成 | E 会場 |

26 日

- | | | |
|----------------------|---|------|
| (3) 新崎盛敏 (東 大・農) | 緑藻カサノリ目 Dasycladales 植物の体形成と属間の類縁関係について | F 会場 |
| (4) 奥野春雄 (京 都 工 繊 大) | 電子顕微鏡立体写真撮影法によるケイ藻殻微細構造の研究 | E 会場 |

10 月 26 日 (日)

トピック 1 亜種と変種について

9.00—12.00 (B会場)

座　長　原　　寛　(東大・理)

- (1) 木村陽二郎 (東大・教養) 高等植物における亜種と変種
- (2) 北村四郎 (京大・理) 亜種と変種について
- (3) 小林義雄 (科学博物館) 菌類における種以下のランク
- (4) トミタ直樹 (京大・理) 細胞遺伝学から見た亜種と変種

トピック 2 核内要素の微細構造と化学

9.00—12.00 (C会場)

座　長　新家浪雄 (京大・理)

- (1) 重保直樹 (奈良女子大・理) 核および染色体の構造の電子顕微鏡的知見
- (2) 太田敬久 (名大・理) 紡錘体および隔膜形成体の細胞化学
- (3) *水野忠孝・山崎典子 (慶応大) Heterochromatin というもの
- (4) 石田政弘 (京大・理) 植物細胞核のDNA

トピック 3 植物の窒素代謝をめぐる諸問題

9.00—12.00 (A会場)

座　長　森　健　志　(名大・理)

- (1) 服部明彦 (東大・応微研) クロレラの窒素代謝について
- (2) 堀田康雄 (名大・理) ベニシダの前葉体初期における形態分化と核酸との関連について
- (3) *太田行人・高田健三 (名大・理) 発芽期植物における核たんぱくの存在様式

10 月 27 日 (月)

トピック 4 植物群落をどのように考えて研究したらよいか

9.00—12.30 (B会場)

座　長　小清水卓二 (奈良女子大・理) ・神保忠男 (東北大・理)

総合討論座長 飯　泉　茂 (東北大・理) ・山根銀五郎 (鹿児島大・文理)

森下正明 (九大・理)

- (1) 鈴木時夫 (大分大・学芸) 標徴種による植物群落の研究
- (2) 沼田真 (千葉大・文理) 群落統計の問題点
- (3) 吉良竜夫 (大阪市大・理工) 実験群落による研究
- (4) 門司正三 (東大・理) 生産構造について

総　合　討　論

トピック 5 不和合性の問題

9.00—12.00 (C会場)

座　長　田中　信　徳　(東大・理)

- (1) 武丸恒雄 (岡山大・理) 菌類の不和合性因子
- (2) 木村劫二 (岡山大・理) 菌類の不和合性現象に関与する変異因子
- (3) 柴田寛三 (東京農大・茂原分校) アブラナおよびダイコン亜種の不和合性について
- (4) 岡部作一 (広島女子短大) 頭花植物、とくにキク科における不和合性遺伝子の性質

トピック 6 隠花植物における形態形成

9.00—12.00 (A会場)

座　長　猪野俊平 (岡山大・理)

- (1) 中沢信午 (山形大・文理) フークス科藻類の実験形態学
- (2) 新崎盛敏 (東大・農) カサノリ類 *Acetabularia* の体形成、とくに栄養枝、胞子枝の変動性について
- (3) 猪野俊平・*西林長朗 (岡山大・理) 藻類の初期発生と器官発生との比較考察
- (4) 野口彰 (熊本大・理) センタイ類における胞子発芽と葉の再生
- (5) *原田市太郎・*伊藤道夫・菅井道三 (名大・理) モエジマシダの配偶体の形成、とくに細胞分化と相関関係について

A 会場 (生理)

		座 長	福岡女子大	吉 岡 俊 一		
9.30	9.43	(A 1)	人工培地によるマメの組織培養法	農林省・林試 [田中・林試]	石川 広 隆 長谷川 正 男	
9.45	9.58	(A 2)	トウモロコシの根の組織培養について	農林省・林試 [田中・林試]	石川 広 隆 長谷川 正 男	
10.00-10.13	(A 3)	トウモロコシの芽ばえにおいてアンモニアの同化におよぼすKイオン効果	広島大・理	林 京 己		
10.15	10.28	(A 4)	ミトリササゲ子葉から芽ばえへの陽イオンの輸送	名 大・理	岡 本 尚	
		座 長	農 技 研	相 見 霊		
10.30	10.43	(A 5)	リン酸イオンのりん酸の吸収と必須カチオンとの関係	大阪府大・教養	小 島 五 男	
10.45	10.58	(A 6)	うるち、もちおよび糖質トウモロコシの胚乳の pH, 無機りんなどの消長	{大阪学芸大 池田分校	井 上 泰 三 田 中 昌 高	
11.00	11.13	(A 7)	植物のいつ分泌中の P ³² 化合物	{金沢大・理 " "	*玉 井 直 人 西 田 晃 二	
11.15	11.28	(A 8)	りん酸欠乏植物における P ³² 吸収転流と吸水について	{金沢大・理 " " " "	西 田 晃 二 玉 井 直 人 *有 馬 忠 雄	
		座 長	大阪市大・理工	永 井 進		
11.30	11.43	(A 9)	葉面吸収後の物質の移行経路について	{九 大・農	前 田 敏 敏 小 島 均 三	
11.45	11.58	(A 10)	作物の根による放射性りん酸の吸収について	{農 技 研 " "	*相 見 霊 村 上 三 高	
12.00	12.13	(A 11)	細胞内凍結を阻止するための要因	北大・低温研	照 本 勲	
12.15	12.28	(A 12)	植物葉片の凍結曲線に現われる耐寒型と非耐寒型	広島大・理	本 田 稔	

< 綜 合 討 論 >

B 会場 (生理・生化)

		座 長	九大・教養	山下 知 治		
9.30	9.43	(B 1)	Conidia の塊りを形成する放線菌 No. 602 株について	大阪学芸大 平野 分校	*川 戸 峯 子 信 夫 隆 治	
9.45	9.58	(B 2)	放線菌の分類に用いられる生理学的特徴の再検討	大阪学芸大 平野 分校	信 夫 隆 治	
10.00-10.13	(B 3)	草花菌の suspension のナタネ幼植物の発育におよぼす影響	千葉大・教養	山 田 保		
10.15-10.28	(B 4)	根粒菌の <i>Rhizobium</i> のマメ科植物の根粒形成におよぼす作用	鹿兒島大・文理	*山 根 銀 五 郎 東 四 郎		
		座 長	熊本大・理	石 川 重 夫		
10.30-10.43	(B 5)	春まきコムギ発芽期の呼吸系について	北 大・理	*石 川 敏 宏 寺 岡 正 一 宇 佐 美 正 男		
10.45-10.58	(B 6)	水稻発芽期の末端呼吸酵素について	北 大・理	*桑 山 弥 寿 男 宇 佐 美 正 一 仲 尾 正 一 宇 佐 美 正 一		
11.00-11.13	(B 7)	細菌のフェノール酸化系について	北 大・理	鈴 木 昇		
11.15-11.28	(B 8)	<i>Azotobacter</i> によるヒドラジンの酸化	福岡女子大			
		座 長	京 大・理	芦 田 讓 治		
11.30-11.43	(B 9)	<i>Acetobacter suboxydans</i> の末端呼吸系について	名 大・理	*吉 家 や す 子 森 健 秀 一 岩 崎 健 一 森 井 健 志 岩 崎 志 寿		
11.45-11.58	(B 10)	腸炎素反応におけるチトクロームの関与について	名 大・理			
12.00-12.13	(B 11)	硝化菌のチトクロームの交換現象について (II)	名古屋大・教養			
12.15-12.28	(B 12)	<i>Thiobacillus thiooxidans</i> のいおう酸化	名 大・理			

< 綜 合 討 論 >

C 会場 (生態)

座 長 横浜市大・文理 福島 博

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|---------------------|---|----------|-----------|
| 9.30- 9.43 (C 1) | 相模湾沿岸の海藻群落 | 三重県立大・水産 | 谷 口 喜 作 |
| 9.45- 9.58 (C 2) | ミツイシコンブの生態学的研究 (II), 生長と寿命とについて | 北 水 研 | 長 谷 川 雄 雄 |
| 10.00-10.13 (C 3) | 池沼における水質の季節的变化と <i>Trachelomonas</i> 属藻類の消長 | 京 大・理 | 日 下 部 有 信 |
| 座 長 金 沢 大・理 津 田 道 夫 | | | |
| 10.15-10.28 (C 4) | 青森県東南部更新世泥炭の花粉分析と植物遺体 | 東北大・理 | 田 島 寛 吉 |
| 10.30-10.43 (C 5) | 後氷期の花粉分析的研究 II, 北アルプス地帯 | 大阪市大・理工 | 塚 田 松 雄 |

< 綜 合 討 論 >

座 長 大阪市大・理工 三 木 茂

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|----------------------|-----------------------------|----------------|-----------|
| 10.55-11.08 (C 6) | 北海道渡島離島小島の植物相 (予報) | 北海道学芸大
函館分校 | 宗 像 和 彦 |
| 11.10-11.23 (C 7) | 九州中部山岳のマツ型森林の分布 | 北海道林試 | 栗 島 博 一 郎 |
| 11.25-11.38 (C 8) | 奈良若草山の植物群落 2. ススキーワラビ群落について | 大分・都野小 | 北 川 昌 典 |
| | | 奈良女子大・理 | 小 崎 孝 卓 |
| | | " | 青 木 正 直 |
| | | " | 中 村 浩 一 |
| 座 長 大 分 大・学芸 鈴 木 時 夫 | | | |
| 11.40-11.53 (C 9) | 結枯山森林の生態調査報告 (第2報) | 東 大・理 | 宮 城 英 夫 |
| | | " | 佐 伯 敏 郎 |
| | | " | 佐 伯 敏 郎 |
| 11.55-12.08 (C 10) | タイ国森林の概観 | 大阪市大・理工 | 小 川 房 人 |
| | | " | 依 田 恭 三 |
| 12.10-12.23 (C 11) | タイ国モンスーン林の構造と組成 | 大阪市大・理工 | 依 田 恭 三 |
| | | " | 小 川 房 人 |

< 綜 合 討 論 >

D 会場 (形態・細胞・分類)

座 長 佐賀大・文理 藤 原 勲

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|--------------------|----------------------------------|----------------|-----------|
| 9.50- 9.53 (D 1) | 石灰岩地帯の植物群における染色体数の問題 | 京 大・理 | 高 木 建 美 |
| 9.45- 9.58 (D 2) | イオハナノエシレイソウにおける胚内分化の機構と要因 | 北海道学芸大
函館分校 | 白 林 正 尚 |
| 10.00-10.13 (D 3) | カモジグサの生態型およびカモジグサとオオタチカモジグサの自然雑種 | 遺 伝 研 | 阪 本 寧 男 |
| 座 長 三重大・学芸 及 川 公 平 | | | |
| 10.15-10.28 (D 4) | タデ属植物の発生学的研究 1 | 遺 伝 研 | 土 井 田 幸 郎 |
| 10.30-10.43 (D 5) | ハウセンカの胚嚢発生について | 愛知女子大 | 西 藤 静 代 |
| 10.45-10.58 (D 6) | ソラメ胚発生の組織化学的研究 | 名 大・理 | 高 尾 昭 夫 |
| 11.00-11.13 (D 7) | ゴマの双生葉形成について (続報) | 東京都大・理 | 堀 順 |

< 綜 合 討 論 >

座 長 名 大・理 原 田 市 太 郎

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|--------------------|-----------------------------------|----------------|---------|
| 11.30-11.43 (D 8) | ユリ科植物の花粉発芽とアミノ酸について | 北海道学芸大
旭川分校 | 沢 田 義 康 |
| 11.45-11.58 (D 9) | オジギソウの細胞生理学的研究 (第10報) | 東京女子大 | 馬 山 芳 雄 |
| 12.00-12.13 (D 10) | ジャガイモのカルス形成の際における組織呼吸の阻害と細胞分裂について | 京 大・理 | 馬 場 三 吾 |
| 12.15-12.28 (D 11) | 酵母菌の出芽とX線感受性について | 立教大・理 | 山 崎 照 俊 |

< 綜 合 討 論 >

A 会場 [生理]

座 長 京大・農小西通夫

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|-------------|-------|----------------------------|----------------|---------------|
| 13.30-13.43 | (A13) | オーキシン作用における原形質表層のリボ核酸の意義 | 大阪市大・理工 | 増田芳雄 |
| 13.45-13.58 | (A14) | 微生物の生長におよぼすインドール酢酸の影響 | 神戸大・理 | 須田省三 |
| 14.00-14.13 | (A15) | インドール酢酸の生長促進作用におよぼす鉄イオンの影響 | { 東大・教養
" " | 柴岡弘郎
*八巻敏雄 |
| 14.15-14.28 | (A16) | イネ子葉鞘のインドール酢酸酸化酵素 | { 東北大・理
" " | *和長尾昌司之 |

座 長 東大・教養 八巻敏雄

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|-------------|-------|--|---------------|--------------|
| 14.30-14.43 | (A17) | 1. インドロキサントノール(1)の生理的意義と作用型について | 京大・理 | 勝見允行 |
| 14.45-14.58 | (A18) | α -ナフトエ酸のハロゲンならびにニトロ基置換体の伸長およびカルス形成作用 | { 京大・理
" 農 | *加藤次郎
三哲夫 |
| 15.00-15.13 | (A19) | ジベレリン酸と抗オーキシン物質との相互作用 | 京大・理 | 加藤次郎 |
| 15.15-15.28 | (A20) | ジベレリンによる根の伸長促進 | 農技研 | 村上浩 |

座 長 奈良女子大・理 小清水卓二

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|-------------|-------|------------------------------|--------------------------|-----------------|
| 15.30-15.43 | (A21) | エンドウ幼茎の伸長におよぼすジベレリンの効果 | { 京大・理
" " | *依田静子
芦田通治 |
| 15.45-15.58 | (A22) | えん麦子葉鞘切片の伸長におよぼすジベレリンと生長素の作用 | { 京大・農
大阪エレクトロニクス工業KK | *小崎山登世
今村駿一郎 |
| 16.00-16.13 | (A23) | アサガオわい性品種によるジベレリンの定量 | { 京大・農
" " | *小川幸光
小田持郎 |

<総合討論>

座 長 農技研 村上浩

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|-------------|-------|---|---------------|----------------|
| 16.30-16.43 | (A24) | 種子発芽におよぼすクマリン類縁物質の作用 | 熊本大・理 | 石川重夫 |
| 16.45-16.58 | (A25) | 担子菌子実体の生長を促進する作用物質の研究 I. ツクリタケ (<i>Agaricus bisporus</i> (Lange) Sing.) の子実体生長促進物質の存在 | { 京大・農
" " | *萩本宏夫
小西通夫 |
| 17.00-17.13 | (A26) | 担子菌子実体の生長を促進する作用物質の研究 II. ツクリタケ (<i>Agaricus bisporus</i> (Lange) Sing.) の子実体存在するオーキシンについて | { 京大・農
" " | *小萩本宏夫
萩本通夫 |

<総合討論>

B 会場 [生理・生化]

座 長 大阪市大・理工 柳島直彦

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|-------------|-------|--|----------------------|---------------|
| 13.30-13.43 | (B13) | 放線菌のこんにやく培地上の発育およびこんにやくマンナンの分解について | お茶の水大・理 | 今井百里江子 |
| 13.45-13.58 | (B14) | <i>Phytophthora infestans</i> (Mont.) Da Bary 菌の胞子形成について | { 島根農大
" " | *山本昌木
谷野淳一 |
| 14.00-14.13 | (B15) | 好ちよう糸状菌の研究 金属鉄研磨面における発芽について | お茶の水大・理 | 大槻虎男 |
| 14.15-14.28 | (B16) | 大腸菌の lysed-protoplast による酵素形成と核酸塩基 analogues の作用 | 阪大・理 | 尾辻望 |
| 14.30-14.43 | (B17) | イースト細胞におけるたんぱく質の代謝的安定性とたんぱく合成との関連 | 名大・理 | 堀田康雄 |
| 14.45-14.58 | (B18) | 種子たんぱく質における種属特異性 | 京大・理 | 桃谷好英
三男吉男 |
| 15.00-15.13 | (B19) | こうじかびのミトコンドリアの酸化に伴なうりん酸エステル化反応 | { 阪大・理
" "
" " | *巖今東奥
山本貴一 |

<総合討論>

座長 岡山大・理 藤 茂 宏

- 15.30-15.43 (B20) 変形菌の ATP 含量と 2,3 の生理的条件下
にわたるその増減について (III) 阪 大・理 奈 野 節 司
- 15.45-15.58 (B21) 緑葉における光化学的リノ酸転移反応 (5)
光化学的還元物質の生成との関連 {東 大・応微研 三 井 旭
東 大・理 太 田 久彦
東 大・応微研 服 部 明彦
" 藤 田 善彦
- 16.00-16.13 (B22) ラン藻および紅藻の phycobilin pig-
ments について 東 大・理 代 谷 次 夫
- 16.15-16.28 (B23) タバコの葉におけるポリフェノールオキシ
ダーゼの誘導について 東 大・理 代 谷 次 夫

座長 九大・農 小 島 均

- 16.30-16.43 (B24) タマネギの炭水化物に関する生化学的研究
(I) {東京教育大・理 藤 沢 敬 一
" 三 輪 知 雄
- 16.45-16.58 (B25) 高等植物の光変阻害 ダフネチンの関与す
る黒変現象 東邦大・理 代 谷 康
- 17.00-17.13 (B26) コナギ子葉に含まれる糖質について {東 大・理 服 部 章 夫
" 堀 中 信 夫
" 堀 部 静 夫
- 17.15-17.28 (B27) ハッシュウマメに存在する一新アミノ酸 {東 大・理 堀 部 静 夫
" 阿 部 敏 夫

<綜 合 討 論>

C 会 場 [細胞]

座長 京大・理 新家 浪雄

- 13.30-13.43 (C12) シャジクモ類の細胞学的研究 VII オオシ
ャジクモ (*Chara corallina*) の造精器の成
熟度と分裂ひん度との関係および分裂ひん
度の日中変化 立 教 大 佐々木 正 人
- 13.45-13.58 (C13) スミレモ科植物の細胞学的観察 和歌山大・学芸 末 松 四 郎
- 14.00-14.13 (C14) 藻類べん毛の mastigonemes の電顕像 愛知学芸大 神 谷 平
- 14.15-14.28 (C15) 植物細胞のオスミウム固定についての電
子顕微鏡的研究 奈良女子大・理 左 貝 あ い 子

座長 東京教育大・理 植田 利喜造

- 14.30-14.43 (C16) 凍結乾燥による植物の組織化学的研究 {京 大・理 三 木 寿 子
" 横 村 岸 秀 夫
" 山 岸 秀 夫
- 14.45-14.58 (C17) 凍結真空乾燥法による *Spirogyra ellipso-*
spora の固定 京 大・理 山 岸 秀 夫
- 15.00-15.13 (C18) *Vicia faba* の根端細胞核中の DNA 量に
ついて 京 大・理 横 村 英 一
- 15.15-15.28 (C19) 'Feulgen 反応-fuchsin 抽出法' によ
る desoxyribo 核酸の微量定量 京 大・理 平 岡 俊 佑

座長 東大・理 和田 文吾

- 15.30-15.43 (C20) こあつもり染色体の DNase 処理 慶 応 大 山 崎 典 子
- 15.45-15.58 (C21) 数種緑藻の pyrenoid の構造について 奈良女子大・理 植 田 勝 己
- 16.00-16.13 (C22) 葉緑体の発達に関する電子顕微鏡的研究 {東京教育大・理 植 田 利 喜 造
" 村 上 悟 悟
京 大・理 新 家 浪 雄
奈良女子大・理 植 田 勝 己
- 16.15-16.28 (C23) Cyanophyta 細胞の構成についての一考察 京 大・理 新 家 浪 雄

座長 名大・理 島 村 環

- 16.30-16.43 (C24) フラスモの電子顕微鏡的研究 阪 大・理 寺 田 保
- 16.45-16.58 (C25) 電子顕微鏡による変形菌の微細構造に
関する研究 (III) 阪 大・理 寺 田 保
- 17.00-17.13 (C26) 紡錘体の電顕像と有糸分裂機構 {東 大・理 佐 藤 文 吾
" 佐 藤 正 一

<綜 合 討 論>

D 会 場 (形態・分類・地理)

		座 長	東京教育大・理	伊 藤 洋		
13.30-13.43	(D12)	電子顕微鏡による針葉樹花粉膜の微細構造	大阪市大・理工	上 野 実 恵		
13.45-13.58	(D13)	圧縮変形されたミツガシワ遺体の復元法	大阪市大・理工	粉 川 昭 平		
14.00-14.13	(D14)	遺体からみた邦産裸子植物	大阪市大・理工	三 木 茂		
14.15-14.28	(D15)	ヘラシダ属 <i>Diplazium</i> の有性世代と 2, 3 の分類学的性質について	成 城	川 崎 次 男		
		座 長	東 大	本 田 正 次		
14.30-14.43	(D16)	日本海諸島植物分布の概要, その三	函館植物研	菅 原 繁 蔵		
14.45-14.58	(D17)	アフガニスタン野生植物 (カラコラム・ヒンズークシ探検隊調査報告)	京 大・理	北 村 四 郎		
15.00-15.13	(D18)	欧亜大陸の東西栽培植物の交流 (カラコラム・ヒンズークシ探検隊調査報告)	京 大・理	北 村 四 郎		
		＜綜 合 討 論＞				
		座 長	服 部 植 研	服 部 新 佐		
15.25-15.38	(D19)	Jungermanniaceae 植物の油体	福岡・修猷館高	尾 川 大 録		
15.40-15.53	(D20)	琉球列島のセン類 III. Mniaceae (チョウナンリ科) の生態・分布	鹿児島大・文理	新 敏 夫		
15.55-16.08	(D21)	日本産カサゴケ科セン類にみられる分布型について II. カサゴケ亜科 (Bryoideae) について	京大・学芸	越 智 春 美		
16.10-16.23	(D22)	本邦産ハリミズゴケ類の分類と分布	広島大・理	鈴 木 兵 二		
		座 長	東京教育大・理	印 東 弘 玄		
16.25-16.38	(D23)	<i>Saprolegnia parasitica</i> について	東邦大・理	砂 山 真 理 子 (旧姓大久保)		
16.40-16.53	(D24)	<i>Prototheca</i> 属に関する研究	長 尾 研	曾 根 田 正 己		
16.55-17.08	(D25)	<i>Oedocephalum</i> 属の子嚢世代について	長 尾 研	椿 啓 介		
17.10-17.23	(D26)	南九州および琉球列島に産するイネ科植物さび菌について	東京教育大・理	平 塚 利 子		

＜綜 合 研 究＞

A 会場 (生理)

座 長 宮崎大・学芸 中山 至 大

- 13.00-13.13 (A27) アサガオ子葉の日長感応に対する弱光の影響 {京大・理 〃} *滝田 勝彦
 13.15-13.28 (A28) 花芽形成の光可逆性に対する kinetin の効果 京大・農 奥田 光郎
 13.30-13.43 (A29) 茎の極性発現、とくに発根に関する一考察 東京学芸大 小林 万寿男
 13.45-13.58 (A30) 花芽分化期および開花期における上展水分条件の変化に伴うダイズの水分生理 下関商高 賀 来 章 輔

座 長 京大・農 今村 駿一郎

- 14.00-14.13 (A31) シロサキツユクサの開花における花卉の行動 兵庫農大 堀 江 格 郎
 14.15-14.28 (A32) 花芽形成の光可逆性に対する kinetin の効果 {宮崎大・学芸 〃} *飛田 博至 温大雄
 14.30-14.43 (A33) 光刺激による胚軸の生長抑制とそれによらばす薬物の影響 徳島大・工 中 山 至 大
 14.45-14.58 (A34) シュウカイドウの無性芽形成(第4報) 日長処理に伴う生長促進および抑制物質の消長 京大・理 藤 井 良 平
 15.00-15.13 (A35) シュウカイドウの無性芽形成(第4報) 日長処理に伴う生長促進および抑制物質の消長 {東北大・理 〃} *江 刺 洋 司
 長 尾 昌 之

<綜 合 討 論>

座 長 京大・理 久世 源太郎

- 15.30-15.43 (A36) シャジクモの膜電位を構成する2種の起電力について 東北大・理 小 田 健 二
 15.45-15.58 (A37) シャジクモの活動電位の伝導速度 {東北大・理 〃} *柴 岡 孝 雄
 16.00-16.13 (A38) シャジクモ節部の伝達の機作 東北大・理 柴 岡 孝 雄

座 長 東北大・理 柴 岡 孝 雄

- 16.15-16.28 (A39) シャジクモ類の原形質分離と原形質流動 {東大・教養 〃} 林 俊 郎
 16.30-16.43 (A40) Chara の節間細胞の電位特性と原形質流動 阪大・理 *上 坪 英 治
 16.45-16.58 (A41) 原形質流動と SH 阪大・理 岸 本 卯 一 郎
 阿 部 重 美

<綜 合 討 論>

B 会場 (生理)

座 長 大阪市大・理工 高 田 英 夫

- 13.00-13.13 (B28) 異常環境下における霊菌の行動 和歌山大・学芸 香 山 時 彦
 13.15-13.28 (B29) 海藻の呼吸と塩分濃度との関係 下関・水産講習所 尾 形 英 二
 13.30-13.43 (B30) 酵母菌の水分生理学的研究 I. 塩添加水度傾斜環境における発育について 広島大・理 高 見 伸 治
 13.45-13.58 (B31) 酵母細胞膜の諸性質について 学 書 院 貝 原 友 次 郎

座 長 神戸大・理 須 田 省 三

- 14.00-14.13 (B32) 酵母に対する 2, 4-D の影響 島根大・文理 西 上 一 義
 14.15-14.28 (B33) 酵母の銅抵抗性の遺伝 {京大・理 〃} *瀬 野 惺 治
 14.30-14.43 (B34) 酵母の銅抵抗性を規定する要因について 甲南大・理 荒 勝 豊
 14.45-14.58 (B35) 銅耐性酵母の有機酸およびアミノ酸代謝 {愛媛大・文理 〃} *村 山 徹 郎
 芦 田 徹 治

<綜 合 討 論>

座長 東大・応微研 渡辺 篤

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|-------------------|--|-------------------------|--|
| 15.15-15.28 (B36) | 銅耐性酵母の硫酸および亜硫酸還元系について | {京大・理
岐阜大・学芸
京大・理 | *菊内 池 忠 寿
内 貴 信 夫
菊 池 忠 夫
内 貴 信 夫 |
| 15.30-15.43 (B37) | S ³⁵ O ₄ による銅耐性酵母のいおう代謝の研究 | {岐阜大・学芸
京大・理
" " | *内 貴 信 夫
菊 池 忠 夫
内 貴 信 夫 |
| 15.45-15.58 (B38) | 酵母のカドミウム耐性 | {甲南大・理
京大・理 | *中 村 運
内 貴 信 夫
内 貴 信 夫 |
| 16.00-16.13 (B39) | 酵母のW変異の誘導, とくにリボ核酸との関係 | 大阪市大・理工 | 柳 島 直 彦 |

座長 東北大・理 長尾 昌之

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|-------------------|-----------------------------------|-----------------|--------------------------------|
| 16.15-16.28 (B40) | 酵母食塩抵抗菌における原形質表層リボ核酸と色素・イオンの透入 I | {大阪市大・理工
" " | *徳 野 真 一
坂 本 敏 彦
高 田 英 夫 |
| 16.30-16.43 (B41) | 酵母食塩抵抗菌における原形質表層リボ核酸と色素・イオンの透入 II | 大阪市大・理工 | 高 田 英 夫 |
| 16.45-16.58 (B42) | マンガン化合物による呼吸欠損酵母菌の誘導について | 大阪市大・理工 | 永 井 進 |

< 総 合 研 究 >

C 会 場 (形態・細胞・分類)

座長 慶応大 水野 忠 敦

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|-------------------|-----------------------------|--------|---------|
| 13.00-13.13 (C27) | モエジマシダ前葉体の細胞分化と造精器形成との関係 | 名大・理 | 伊 藤 道 夫 |
| 13.15-13.28 (C28) | モエジマシダ初期前葉体における細胞の分裂と伸長との関係 | 名大・理 | 菅 井 道 三 |
| 13.30-13.43 (C29) | シダの原系体における“頂化”と“基化” | 山形大・文理 | 中 沢 信 午 |

座長 遺伝研 竹 中 要

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|-------------------|---|---------|---------|
| 13.45-13.58 (C30) | ワタナバソウの染色体について | 大分大・学芸 | 三 宮 正 信 |
| 14.00-14.13 (C31) | タンポポ類の核型分析 (4報) | 東京都大・理 | 西 岡 泰 三 |
| 14.15-14.28 (C32) | <i>Takakia lepidozoides</i> の染色体とセンタイ類染色体進化の一考察 | 広島大・理 | 辰 野 誠 次 |
| 14.30-14.43 (C33) | オオムギに見出だされた long chromosomes | 木 原 生 研 | 土 屋 工 |

座長 広島大・理 辰 野 誠 次

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|-------------------|---|--------|---------|
| 14.45-14.58 (C34) | 九州地方産 <i>Kalimeris incisa</i> 群の染色体数と地理的分布について | 熊本大・理 | 井 上 覚 |
| 15.00-15.13 (C35) | アゼトウナ雑種集団の核型の変異 | 東京都大・理 | 酒 井 文 三 |
| 15.15-15.28 (C36) | エンレイソウ (<i>Trillium smallii</i> Maxim.) の1集団における染色体組成の分析 | 北大・理 | 佐 保 貴 |

座長 神戸大・理 広 瀬 弘 幸

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|-------------------|--|-----------------|----------------------------|
| 15.30-15.43 (C37) | <i>Chroococcus</i> (ラン藻) の分類 | {横浜市大・文理
" " | *福 島 博
丸 山 晃
小 林 博 子 |
| 15.45-15.58 (C38) | <i>Cymbella sumatrensis</i> Hustedt (ケイ藻) について | {横浜市大・文理
" " | *福 島 博 子
小 林 博 子 |
| 16.00-16.13 (C39) | 日本産シャジクモ類 第12報(再びホシツリモについて) | 東京都大・理 | 加 崎 英 男 |

座長 長崎大・水産 岡 田 喜 一

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|-------------------|---|-----------------|---------------------|
| 16.15-16.28 (C40) | 九州産緑色カワモズクの2,3について | 熊本・宇土高 | 森 通 保 |
| 16.30-16.43 (C41) | 日本産カワモズク <i>Batrachospermum moniliforme</i> Roth の生活史について | {神戸女学院
神戸大・理 | *瀬 戸 良 三
広 瀬 弘 幸 |

座 長 北大・理 山田 幸 男

16.45-16.58 (C42)	カキノリ科植物の生活環—とくにタマイタダキとヒロハタマイタダキを中心として	東京教育大・理	千 原 光 雄
17.00-17.13 (C43)	日本海の海藻 I. 佐渡島の海藻	新潟大・理	野 田 光 藏
17.15-17.28 (C44)	藻類にみられる遊走細胞の放出孔の構造とその繁殖関係的な意味	神戸大・理	広 瀬 弘 幸

D 会 場 (生 態)

座 長 東大・理 高 橋 基 生

13.00-13.13 (D27)	森林植物の根の分布と土壌中の根の観察	広島 県	森 千 春
13.15-13.28 (D28)	シバ種子の休眠と外被	九大・農	高 木 正 元
13.30-13.43 (D29)	放牧地における牛馬ふん上の植群	{ 東北大・理 宮城・岩ヶ崎高	* 藤 田 重 悦

座 長 千葉大・文理 沼 田 真

13.45-13.58 (D30)	植物の根群	北海道学芸大 釧路分校	田 中 隆 徳
14.00-14.13 (D31)	植物の根の分布と土壌中の根の観察	大阪市大・理工	近 宿 廉 也

座 長 大阪市大・理工 吉 良 竜 夫

14.15-14.28 (D32)	常緑広葉樹の茎および根の呼吸ならびにその幼木の葉・茎・根の重量比について	鹿児島大・教育	楠 元 司
14.30-14.43 (D33)	環境変化(温度・水分その他)と根系呼吸	{ 東大・理 三菱鉱業研	* 高 橋 基 生 渡 辺 庄 美
14.45-14.58 (D34)	越冬麦の生育経過と根系呼吸	東大・理	高 橋 基 生

< 綜 合 討 論 >

座 長 東北大・理 神 保 忠 男

15.10-15.23 (D35)	草原植物の根の分布と土壌中の根の観察	九大・理	小 谷 信 次
15.25-15.38 (D36)	草原植物の根の分布と土壌中の根の観察	{ 九大・理 " " " "	* 小 村 精 矢 小 谷 信 次 田 川 隆 英
15.40-15.53 (D37)	草原植物の根の分布と土壌中の根の観察	{ 九大・理 " "	* 細 川 隆 英 小 村 精 矢

座 長 岡山大・農生研 高 須 謙 一

15.55-16.08 (D38)	石灰植物分布についての一考察(V)(炭酸石灰、置換性石灰およびN/5 塩酸浸出 Ca と植生との関係)	広島大・教育 福山分校	寺 尾 茂 美
16.10-16.23 (D39)	浸透圧の日変化と気象環境要素との関連	京大・理・気象研	村 田 茂 三

座 長 東大・理 門 司 正 三

16.25-16.38 (D40)	ウキクサの生長と栄養塩濃度との関係	大阪市大・理工	生 嶋 功
16.40-16.53 (D41)	生長量とN施用量間の好適関係の定式化	{ 大阪市大・理工 " " 大阪市大・医	* 藤 田 重 悦 高 橋 基 生 渡 辺 庄 美

< 綜 合 討 論 >

A 会場 [細胞・遺伝]

		座 長	東京大・理	小 野 正 彦		
13.00	13.13	(A 12)	四国産トウキビ在来種の染色体の‘こぶ’	農 技 研	須 藤 千 春	
13.15	13.28	(A 13)	オーストラリア種 <i>Nicotiana gossei</i> とアメリカ種数種との雑種	遺 伝 研	竹 中 要	
		座 長	岡山大・理	木 村 一		
13.30	13.43	(A 14)	ミミズに関する研究	山口・筑毛南高	広 本 一 由	
13.45	13.58	(A 15)	アカパンカビ子実体形成時における雌雄型による NO_3^- および NH_4^+ の消費について	豊田高工大	伊 藤 太 郎	
14.00	14.13	(A 16)	(取消し)			
		座 長	東大・数	佐 藤 重 平		
14.15	14.28	(A 17)	果樹のつき木による栄養雑種 丸形身不知とゴールデンデリシャスの例について	北海道・松前清部小	沢 利 政 俊	
14.30	14.43	(A 18)	秋まき小麦にみられた分枝性異常穂の出現について	北 大・理	増 淵 法 之	

< 綜 合 討 論 >

B 会場 [生 理]

		座 長	お茶の水大・理	大 槻 虎 男		
13.00-13.13	(B43)	微生物によるろう・パラフィンの分解(附) 2,3 のポリマーの炭素源としての意味	愛媛大・文理		官 本 義 男	
13.15-13.28	(B44)	緑藻の swarmer の運動と光	東 大・農		志 平 依 久 子	
13.30 13.43	(B45)	ラン藻の純粋分離法について	広島大・理		中 谷 茂 彦	
13.45-13.58	(B46)	温泉利用によるラン藻の経済的産について	{ 東 大・応微研 " " " "	* 渡 辺 明 彦 服 部 善 彦 藤 田 千 里		
		座 長	東 大・理	服 部 静 夫		
14.00 14.13	(B47)	緑葉の光電反応 (第 2 報) 光の強さと反応経過	東北大・農研		西 崎 友 一 郎	
14.15-14.28	(B48)	葉緑素を欠く一粒系コムギ突然変異体にみられる低温処理による葉緑素形成 (予報)	{ 近畿大・農 京大・数産		* 杉 野 守 介 山 下 孝 介	
14.30-14.43	(B49)	<i>Euglena</i> の葉緑体反応	{ 岡山大・理 " " " "		* 藤 茂 宏 鈴 木 洋 太 郎 佐 藤 公 行	

< 綜 合 討 論 >

C 会場 [生 理]

		座 長	広島大・理	福 田 八 十 楠		
13.00	13.13	(C 45)	柱頭および発芽時の花粉の浸透圧の変化	{ 横浜市大・文理 " "	*岩 波 洋 造 村 岡 武	
13.15	13.28	(C 46)	吸水力におよぼす外液のイオン価の影響	{ 京 大・理 京都工繊大	昌 山 伊 佐 男 *河 野 清	
13.30-13.43	(C 47)		水草の葉の浸透価, Na 含量と透出について	大阪市大・理工	河 原 晨	
		座 長	鹿児島大・文理	山 根 銀 五 郎		
13.45	13.58	(C 48)	植物組織の結合水測定方法について	{ 広島大・理 " "	*高 沖 武 福 田 八 十 楠	
14.00-14.13	(C 49)		植物の浸透価, 結合水および呼吸の水分生理的相互関係	広島大・理	高 沖 武	
14.15	14.28	(C 50)	熱傾斜 (temperature) と水分傾斜 (hydration) との非相似性	{ 広島大・理 " "	*本 田 稔 福 田 八 十 楠	
14.30-14.43	(C 51)		原形質膜の湿度制御機構	広島大・理	福 田 八 十 楠	

< 綜 合 討 論 >

D 会 場 [形 態]

座 長 茨城大・文理 佐 藤 正 己

- | | | | |
|-------------------|--|-----------------|---------------------|
| 13.00-13.13 (D42) | セン類のさく歯の発生学的研究 ヨツバゴケについて | 島根大・文理 | 斎 藤 真 太 郎 |
| 13.15-13.28 (D43) | <i>Nitella inokasiraensis</i> の生長点の分化と器管形成 | { 東京都大・理
" } | 加 崎 英 男
*岩 崎 尚 彦 |
| | 座 長 名大・教養 | 熊 沢 正 夫 | |
| 13.30-13.43 (D44) | アカサ科の一次管束系について | 埼玉大・文理 | 井 上 隆 吉 |
| 13.45-13.58 (D45) | イネの根の組織の発達 | 東 大・理 | 島 袋 敬 一 |
| | 座 長 東 大・理 | 前 川 文 夫 | |
| 14.00-14.13 (D46) | 外巻葉の形成とツツジ科の系統 | お茶の水大・理 | 原 肇 |
| 14.15-14.28 (D47) | らせん葉序の成立についての一つの考え方 | 東京教育大・理 | 伊 藤 洋 |
| 14.30-14.43 (D48) | 双子葉類前葉配列変化に関する統報 | 名大・教養 | 熊 沢 正 夫 |

< 綜 合 討 論 >

(Memo.)

② 評議員会 日時 10月24日(金): 15.30

場所 日活ホテル

② 記念撮影 日時 10月27日(月): 15.15

場所 工学部本館玄関前

② 総会 日時 10月27日(月): 15.30-17.00

場所 工学部大講堂

② 懇親会 日時 10月27日(月): 18.00-20.00

場所 博多帝国ホテル

② 公開講演 (日本植物学会主催, 西日本新聞社後援)

ダーウィン進化論百年記念公開講演会

日時 10月26日(日) 15.30-17.00

会場 福岡市渡辺通り六丁目 西日本新聞社講堂

1. 木村陽二郎: ダーウィンの進化論のなりたち
2. 松浦 一: 日本におけるダーウィニズムの二つの流れ

② 植物学研究連絡委員会

日時 10月26日(日) 12.00-14.30

場所 九州大学理学部会議室

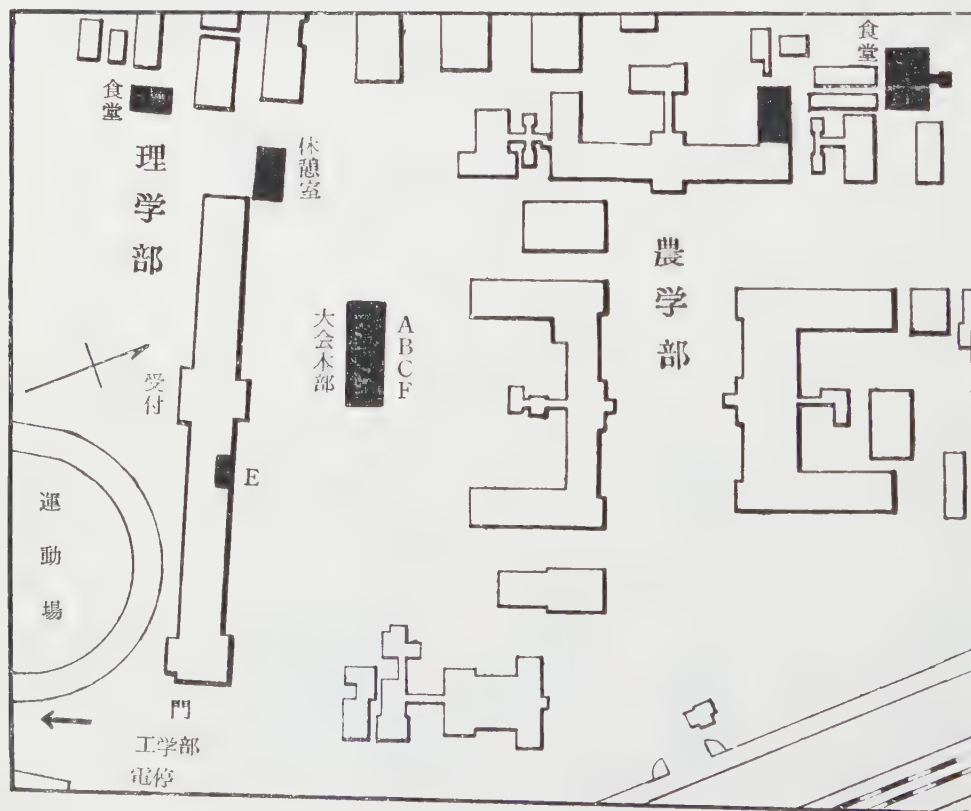
② 被同伴者の市内見学

日時 10月27日(月) 10.00-16.00

集合場所 九州大学理学部玄関前

九州大学農学部・理学部略図

(西鉄市内線・九大前下車)



The Effect of Cobalt on the Growth of Pollen I.

by Yoshio YAMADA*

山田義男*: 花粉の生長におよぼすコバルトの効果 I.

Received July 12, 1958

Oxine (8-hydroxyquinoline) and other chelating agents are of interest because of their possible bacteriostatic and fungicidal properties and their power to chelate metals. Several workers have postulated that the toxicity of oxine to microorganisms may be due to its ability to form chelate complexes with essential trace metals, which are then rendered unavailable for metabolic processes^{1),2)}. Recently Albert *et al.*³⁾ found that there was strong positive correlation between bacteriostatic activity and chelating power in a series of concentrations of oxine, and demonstrated reversal of oxine inhibition by Co for Gram-positive bacteria, and by Zn and Fe for Gram-negative bacteria. Similar effects have been noted in other biological systems involving chelating agents and metals. In an attempt to determine the trace element requirements of mammalian spermatozoa, White^{4),5)} investigated the effect of several chelating agents on motility, and found that Co reduced the spermicidal activity of some chelating agents, while other mixtures of heavy metals and chelating agents were more toxic than the chelating agents alone. Gale⁶⁾ found that oxine inhibited glutamic acid assimilation in *Staphylococcus aureus*, but the addition of Co, Mn and Fe annulled this.

In this paper, the effect of chelating agents and their metal complexes on the growth of pollen has been studied with a view to determining their trace element requirements.

Materials and Methods

In the majority of the experiments reported here *Lilium longiflorum* Thunb. was employed but some other species, viz. *L. auratum* Lindl. and *L. Maximowiczii* Regel, gave essentially similar results. The former was readily procurable in good condition, and usually gave a high percentage of germination under appropriate circumstances. *Lilium* pollen gave its best germination several hours after dehiscence and retained the ability to germinate for 3 days or more. The time allowed for growth was chosen after preliminary experiment on the pollen, and its selection was based on the length attained by the tubes. The experiments were stopped before the tubes grew too long to be measured accurately. Within a growth period of 15 hours for *Lilium* pollen at the optimum temperature there were relatively few tubes longer than

* Biological Institute, Faculty of Liberal Art and Education, Gunma University, Maebashi, Japan. 群馬大学学芸学部生物学教室

the diameter of the low power microscopic field. Accordingly measurements were made at intervals of 15 hours throughout the following experiments. The cultures were grown in an oven at 28°-30° under red lamp. For counts of percentages and measurements of tubes the low magnification of a stereoscopic dissection microscope was used. Fields for study were chosen at random among those containing approximately 50 grains.

Eight per cent sucrose in glass-redistilled water was used as the basal medium. Agar was not added because it is a natural product containing some trace metals. For example, with the use of polarography, the zinc content of agar (R. G.) was about 280 $\mu\text{g./g.}$ dry weight, whereas that of sucrose (R. G.) was negligible. As the amount of trace metals present is a critical factor in chelating agent inhibition, acid-washed glasswares were used to avoid contamination with extraneous metals. The chelating agents used were ethylenediamine-tetraacetate (EDTA) and 8-hydroxyquinoline (oxine) and the metals, copper, cadmium, nickel, zinc, iron, manganese, cobalt, calcium and magnesium, were added as the sulphate salts.

All treatments had 5 replicates, and in each experiment a control culture containing only 8 per cent sucrose was used. In control series, the percentages of germinated grains ranged from 50 to 60 and a mean tube length from 8.0 to 9.0 mm. under present conditions. The percentage range of germination was recorded as follows:

60-80 %	++++
50-60 % (control)	+++
30-50 %	++
10-30 %	+
2-10 %	±
no germination	-

The rate of respiration was measured in Warburg manometers during the periods of 2 hours at 30°. Approximately 10 mg. fresh weight of pollen grains were used in all cases. The volume of the medium, including pollen grains, in each flask was always made up to 2.0 ml.

Results and Discussion

1. Effects of various concentrations of EDTA and oxine on the germination and tube length of *Lilium* pollen.

Table 1 shows measurements made with culture of *L. longiflorum* pollen in various concentrations of EDTA and oxine at 30°. The same tests were made with *L. auratum* and similar results were obtained. In both cases, concentrations of EDTA higher than $5 \times 10^{-3}\text{M}$ were toxic, i. e., caused decreased germination, retarded elongation, and caused bursting and distortion of tubes. Lower concentrations were favorably stimulative with a maximum response in the 10^{-5}M dilution. It will be noted that at 10^{-5}M the average length of the tubes exceeds that in the control. Thus, about 70 per cent germination was found in the 10^{-5}M EDTA series and a mean tube length

Table 1. Effects of various concentrations of EDTA and oxine on the germination and tube length of *Lilium* pollen.

EDTA									
	10 ⁻³ M	2 × 10 ⁻³ M	5 × 10 ⁻³ M	10 ⁻⁴ M	2 × 10 ⁻⁴ M	5 × 10 ⁻⁴ M	10 ⁻⁵ M	5 × 10 ⁻⁵ M	Control
Percentage of germination		+	+	++	++	+++	++++	++++	+++
Mean tube length in mm	2.36	4.38	5.70	6.75	7.74	8.08	10.85	9.34	8.57

Oxine									
Percentage of germination	—	—	—	±	+	++	+++	++++	+++
Mean tube length in mm.	0	0	0	3.14	4.09	5.50	6.32	7.35	7.96

of 10.85 mm., in contrast with about 60 per cent and 8.57 mm. in the control. At 5 × 10⁻⁴M, however, the growth obtained was equal to that in the control. At 10⁻⁴M the average growth of 5 tests was decidedly less than the control, but the toxicity was not so great.

Further, the oxygen uptake of *Lilium* pollen suspensions containing various concentrations of EDTA was compared with that in the control medium. The results are shown in Table 2. The oxygen uptake was markedly enhanced by 10⁻⁵ M and

Table 2. Oxygen uptake by *Lilium* pollen in 8 per cent sucrose solution containing various concentrations of EDTA expressed as percentage of uptake (μl./10 mg. fresh wt./hr.) occurring in the same sucrose solution. Each replicate is for a different pollen sample.

Concentration of EDTA	O ₂ uptake, percentage of control			
	1st hour		2nd hour	
	rep. 1	rep. 2	rep. 1	rep. 2
1 × 10 ⁻³ M	55	83	36	43
5 × 10 ⁻³ M	82	88	42	56
1 × 10 ⁻⁴ M	84	95	78	62
5 × 10 ⁻⁴ M	90	111	107	141
1 × 10 ⁻⁵ M	102	128	143	164
5 × 10 ⁻⁵ M	97	105	115	155

it was strikingly inhibited by 10⁻³M. From these experiments it is clear that there is a close parallelism between the pollen-growth and respiration.

By similar methods, oxine was found to be toxic in a concentration of 10⁻⁴M but was without favorable stimulation in more dilute solutions. The 10⁻⁵M series was essentially similar to the control. However, the percentages of germination were decidedly greater in the presence of 5 × 10⁻⁵M oxine but the respiration and the mean tube length were somewhat less than those in the control.

According to Heath and Clark^{7,8)}, the inhibition of root growth caused by 10⁻⁵M oxine or 10⁻⁵M EDTA alone was largely prevented by the presence of 10⁻¹¹M EDTA in the former case or of 10⁻¹¹M oxine in the latter.

In the present investigation this test was repeated and essentially similar results were obtained. That is, there was evidence of a remarkable mutual antagonism; the pollen growth-inhibitory effect of 10^{-4} M EDTA or 10^{-4} M oxine alone was completely removed by the addition of 10^{-5} M oxine in the former case or of 5×10^{-5} M EDTA in the latter.

Of the two chelating agents, EDTA seemed to have a stronger stimulatory effect on *Lilium* pollen than oxine.

2. Effects of heavy metals on the growth of pollen.

The primary purpose of this section is to present the results of experiments concerning the effect of divalent metals on the growth of pollen. The metals were all added as the sulphate salts.

In a preliminary test with various concentrations of copper it was found that germination is entirely prevented at 10^{-3} M and 5×10^{-3} M as indicated in Table 3.

Table 3. Effects of various concentrations of cupric sulphate on the germination and tube length of *Lilium* pollen.

	10^{-3} M	5×10^{-3} M	10^{-4} M	2×10^{-4} M	5×10^{-4} M	10^{-5} M	2×10^{-5} M	5×10^{-5} M	10^{-6} M	Control
Percentage of germination	—	—	±	±	+	++	++	+++	++++	+++
Mean tube length in mm.	0	0	1.62	2.96	4.22	5.80	6.96	7.63	8.85	8.23

At 5×10^{-4} M, germination was reduced and pollen tube development arrested; almost complete bursting of tubes resulted in this series. At 5×10^{-5} M, however, growth was nearly equal to that in the control. Lower concentrations were favorably stimulative with a maximum response in the 10^{-6} M dilution. At 10^{-6} M about 5 per cent of the tubes attained a length of 11.3 mm. and a mean tube length of 8.85 mm. and about 65 per cent germination was found, in contrast with 8.23 mm. and about 60 per cent in the control.

Other metallic ions (zinc, cadmium, nickel, calcium and magnesium) were tested, and found to have not so marked a stimulating effect. Brink⁹⁾ reports that in the presence of calcium salts in concentrations ranging from 0.02 M to 0.002 M, the growth of sweet pea pollen tubes is markedly enhanced. The writer has attempted to repeat this test, but without success. These heavy metals completely inhibited germination at 5×10^{-3} M. At 10^{-4} M, the average length of the tubes in each test medium was less than half of that in the salt free control medium. At 10^{-5} M, however, growth obtained was equal to that in the control. These heavy metals give average amounts of growth of similar magnitudes.

Manganese and iron ions showed a slight stimulating activity at 5×10^{-4} M but were not further investigated because great variation was noted.

Comparing the effect of cobalt with that of these heavy metals at 10^{-4} M which were found to be highly toxic, it is worthy to mention the finding that cobalt in

corresponding concentrations promoted growth in a very marked degree. As is shown in Table 4, increases in growth were evident in 10^{-4}M , $2 \times 10^{-4}\text{M}$, $5 \times 10^{-4}\text{M}$ and

Table 4. Effects of various concentrations of cobalt sulphate on the germination and tube length of *Lilium* pollen.

	10^{-3}M	$2 \times 10^{-3}\text{M}$	$5 \times 10^{-3}\text{M}$	10^{-4}M	$2 \times 10^{-4}\text{M}$	$5 \times 10^{-4}\text{M}$	10^{-5}M	Control
Percentage of germination	—	±	++	+++	+++	+++	++++	+++
Mean tube length in mm.	0	0.97	5.51	7.23	8.26	11.05	9.64	8.07

10^{-5}M . When cobalt was added in a concentration of $5 \times 10^{-4}\text{M}$, this increase amounted to nearly 40 per cent. Thus, about 75 per cent germination was found in this series and a mean tube length of 11.05 mm., in contrast with about 55 per cent and 8.07 mm. in the control. However, no germination was obtained at 10^{-3}M . At $5 \times 10^{-3}\text{M}$, the average growth of 5 tests proved to be somewhat less than the control, but the toxicity was not so great. On the other hand, the oxygen uptake was abnormally enhanced by $5 \times 10^{-4}\text{M}$, and it was decidedly inhibited by $5 \times 10^{-3}\text{M}$. In the experiment indicated in Fig. 1, comparisons were made of oxygen uptake when the sucrose content of the medium was maintained constant and the cobalt concentration was altered. From these experiments it is clear that cobalt alone seems to exert favorable effect on the growth of *Lilium* pollen; but information from the present experiment does not refer to the physiological role of this metal.

Recently Thimann¹⁰⁾ reported that cobalt (given as chloride) increases the elongation of etiolated pea stem sections in auxin and its effect is almost doubled in the presence of sucrose, and suggested that cobalt promotes some step in oxidative metabolism which normally makes a source of energy (perhaps ATP) available for growth and diverts it from other metabolic roles.

However, further studies will be necessary to decide this possibility, but

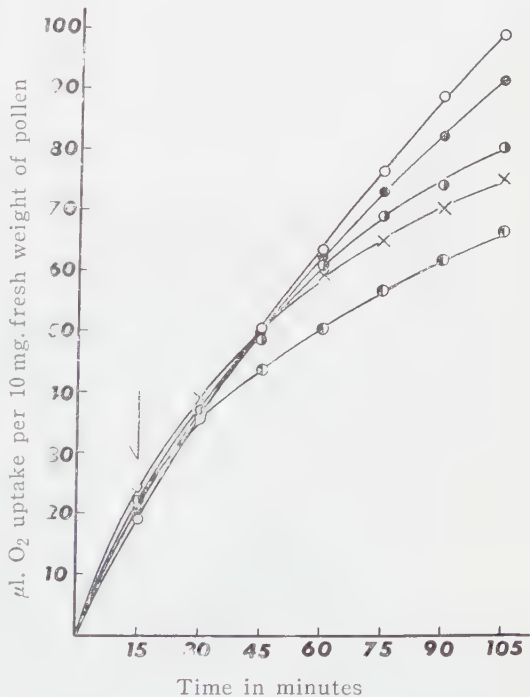


Fig. 1. Effects of various concentrations of cobalt sulphate on the oxygen uptake of *Lilium* pollen.

× — × control, ● — ● 10^{-5}M ,
○ — ○ $5 \times 10^{-4}\text{M}$, ◐ — ◐ 10^{-4}M ,
◑ — ◑ $5 \times 10^{-3}\text{M}$ cobalt sulphate.

Arrow shows time at which the side arm was emptied into respiration flasks.

it is inferred that cobalt is an essential constituent of *Lilium* pollen.

3. Effects of heavy metals on toxicity of chelating agents.

If the growth-inhibitory effect of these chelators is due to their depriving the pollen plasma of essential trace elements, it should be possible to prevent it by adding the appropriate element to the culture medium.

As is stated in above sections, it was found that either chelating agents at $10^{-4}M$ or heavy metals (except cobalt and manganese) at $10^{-4}M$ caused decreases in pollen growth and oxygen uptake of about the same magnitude, 20-40 per cent.

Tests with *Lilium* pollen showed that cobalt reduced the toxicity of EDTA and oxine. Mixtures of other heavy metals and chelating agents were, however, more toxic than the chelating agents alone. Table 5 shows the effects of adding $10^{-4}M$ copper, iron, zinc, cadmium, manganese, nickel and cobalt to *Lilium* pollen in the presence of $10^{-4}M$ EDTA.

Cobalt caused a highly significant decrease in the toxicity of EDTA for the growth of pollen. It had a similar effect on the toxicity of $10^{-4}M$ oxine. However, when cobalt was added in a concentration of $5 \times 10^{-3}M$, and then the rate of respiration was measured, the oxygen uptake was found to be markedly enhanced up to 120 per cent of the control. Similar findings for bacteria have recently been made by Albert *et al.*³⁾.

Copper increased the growth-inhibitory effect of chelating agents. In this series of tests, no germination was obtained.

Furthermore, other metals also increased the growth-inhibitory effect of chelating agents. In these tests, the tubes were extremely crooked and exhibited a strong tendency to burst after 15 hours culture.

Nickel combines more readily than cobalt with most chelating agents.¹¹⁾ If cobalt acts by competing with other heavy metals to form a non-toxic complex⁵⁾, then nickel might be expected to be equally effective. This possibility was examined but without success. There is little doubt that, for the growth of pollen, EDTA and oxine were more toxic in the presence of nickel (Table 5).

Table 5. A comparison of the effect of $10^{-4}M$ heavy metal ions on the toxicity of $10^{-4}M$ EDTA for the growth of *Lilium* pollen.

	Control	EDTA	EDTA plus following metal						
			Copper	Cadmium	Nickel	Zinc	Iron	Manganese	Cobalt
Percentage of germination	+++	++	—	+	+	++	++	+++	++++
Mean tube length in mm.	8.14	5.96	0	0.93	2.18	3.97	5.63	6.72	7.64

Magnesium and calcium were not effective in reducing the toxicity of these chelating agents.

However, manganese was slightly effective in reducing the toxicity of these chelating agents.

From these experiments it is suggested that growth-inhibitory effect on pollen of these chelating agents may be dependent on combination with trace concentration of copper and other heavy metals, and that the antagonistic effect of cobalt may be due to its protecting a certain important chemical machinery.

Summary

1. The effects of chelating agents and their metal complexes on the growth of *Lilium* pollen were investigated.
2. A remarkable mutual antagonism was found in experiment using EDTA and oxine.
3. Cobalt, given as sulphate, was found to enhance markedly the oxygen uptake and the growth of pollen.
4. The toxicity of EDTA and oxine was reduced by the addition of cobalt, and only slightly by manganese.
5. It has been inferred that cobalt and EDTA ($10^{-5}M$) act on promotion of pollen-growth through different mechanisms.
6. It has also been suggested that at least in *Lilium* pollen-growth, cobalt may be an essential trace element.

The writer wishes to express his gratitude to Prof. S. Hori for his kind encouragement and valuable advice throughout this work.

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摘 要

花粉の生長に必須な微量金属要素をきかぬために、キレート試薬、種々の重金属（硫酸塩として与えた）ならずにこれらの金属キレート化合物が花粉粒の発芽、花粉管の伸長および花粉粒の呼吸におよぼす影響をしらべた。用いた材料は主としてテッポウユリ (*Lilium longiflorum*) である。その結果、コバルトのいちじらしい効果を見いだした。

すなわち、Co イオンは単独で花粉の生長にいちじらしい促進効果を示し、同時に酸素消費も明らかに对照よりも増加した。さらに、EDTA, oxine の毒性はコバルトの添加により抑制されるのみならず、むしろ促進効果を示した。なお、マンガンにも多少このような拮抗作用が認められた。これに反して、他の重金属の添加はキレート試薬の毒性をさらに増大させた。

以上のことから、少なくともテッポウユリでは、Co イオンはその生理的機能は不明ではあるが、花粉の生長に必須な微量金属要素であると推定される。

Formation of Starch in Isolated Chloroplasts

I. Experiments with Isolated Chloroplasts Obtained by Ultrasonic Treatment*

by Rikizo UEDA**

植田利喜造**：遊離葉緑体のデンプン形成 I. 超音波作用で遊離した葉緑体での実験

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Introduction

As is well known, the chloroplast displays its photosynthetic activity to a limited extent outside the intact cell; namely, the chloroplast suspension evolves an appreciable amount of oxygen under illumination in the presence of leaf extract^{17),18)} or certain oxidants^{5),13),20),21),22),23),24),27),34)} in accordance with the process of Hill-reaction^{20),21),23)}. In relation to this matter, our attention is drawn, however, to an important problem, whether the chloroplast is capable of performing its inherent function of starch formation even in the absence of mother cytoplasm. In order to answer this question, a number of researches have been carried out by several authors^{6),7),8),9),10),11),14),15),16),17),19),24),25),26)}.

Lubimenko²⁸⁾ had succeeded at first in releasing plastids from the other cytoplasmic components by means of tissue-autolysis. The released chloroplasts obtained by him remained green and apparently alive during nine month period. Later, Flint¹²⁾ also obtained, by UV-irradiation of *Azolla*-cell, several isolated chloroplasts, which were still capable of dividing by themselves and also of synthesizing starch in the absence of both nucleus and cytoplasm.

Recently, Arnon (1955) published a paper entitled "The chloroplast as a complete photosynthetic unit," in which he stated that the isolated spinach chloroplasts not only split water to evolve oxygen but also reduce carbon dioxide into carbohydrate^{1),2),3),4)}.

According to the experiments of Thomas *et al.*^{29),30),31)} carried out on suspension of *Spirogyra* chloroplasts, the rate of photosynthesis was found to be quite similar to that of the chloroplasts in intact cells.

In view of the overwhelming concept that the isolated chloroplast has no capacity

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** Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan. 東京教育大学理学部植物学教室.

of fixing carbon dioxide, it is important for us to carry out further examinations on this matter by means of newly designed experimental procedures.

In 1949, the present author briefly noted his earlier findings, that the chloroplasts isolated into a vacuole of the same cell were, indeed, capable of producing starch grains³²⁾. The experimental technique applied consists in translocating plastids *in situ* into vacuoles by ultrasonic waves³⁵⁾.

The present paper comprises further studies concerning viability and function of the chloroplasts isolated by ultrasonic treatment.

Materials and Methods

Materials used in this experiment were leaf cells of *Elodea densa* and cells of *Spirogyra**, which were made free from starch grains altogether by keeping in the dark. An isolation of the chloroplasts was achieved by following procedure:

Isolation of chloroplasts by means of ultrasonic waves.

As previously reported³⁵⁾, leaf blades of *Elodea* or filaments of *Spirogyra* were put on a thin glass slip and mounted with water and covered with a small piece of cover slip. This was set on a quartz plate vibrator under the microscope (Fig. 1). The vibrator consists of a disk 20 mm. in diameter, 2 mm. in thickness, equipped with a transparent window 5 mm. in diameter at its center. Between the glass slip and the vibrator, an aqueous layer was inserted in order to bring about an efficient transmittance of the ultrasonic waves. The distance between the material and the vibrator was about 1 mm.. The wave length of the ultrason applied was ca. 1 mm.. The waves were applied to the material for 20 to 30 seconds. The intensity of the plate electric current of the apparatus was 50 mA. (200 V).

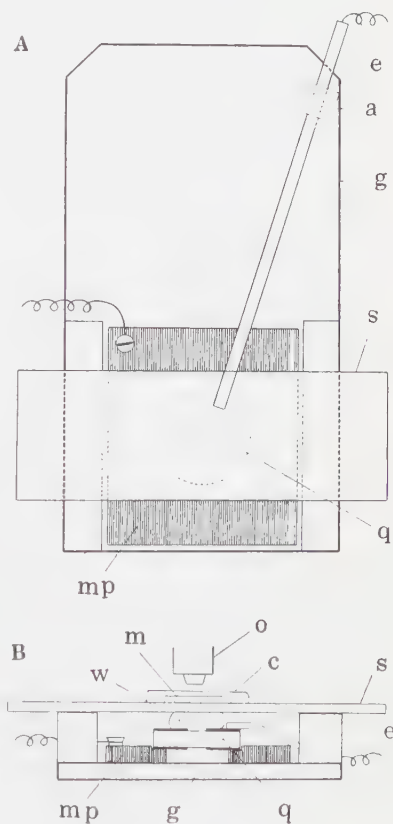


Fig. 1. Diagrammatic representation of the equipment for ultrasonic treatment.

A: surface view. B: lateral view. a: adhesive plaster, e: electric wire, c: cover slip, g: glass plate, m: plant material, mp: metallic plate, o: objective of the microscope, q: quartz plate, s: slide glass, w: water. \times ca. 0.7

* Cell of *Spirogyra* was 92 μ in diameter and 150 to 410 μ in length. Each cell contained five chloroplast bands of spiral form. Cell membrane was covered with jelly-like sheath of 3 μ in thickness.

By appropriate action of ultrasonic waves, a number of solitary chloroplasts*, several aggregates of chloroplasts and the cytoplasmic fragments with or without chloroplasts were liberated from the mother cytoplasm** of *Elodea*-cells and passed into the vacuole. In *Spirogyra*, the chloroplast band was disrupted into fragments, and then transferred into the vacuole.

After ultrasonic treatment, materials (*Elodea* leaves or *Spirogyra* filaments) were cultured in moist chambers by the agar-plate method³³⁾, or else in tap water in glass vessels. Some of the cultures were exposed to diffuse day light through the window and the others were illuminated with fluorescent lamp (20 W) at 20 cm. distance (temperature: $20 \pm 2^\circ$ or $7 \pm 2^\circ$).

The production and consumption of starch grains in chloroplast fragments of *Spirogyra* were also examined under intermittent illumination at $11 \pm 3^\circ$.

The starch grain formed by the isolated chloroplasts was carefully compared in amount under the microscope with that formed by the chloroplast in the mother cytoplasm as well as by the chloroplast in the intact cell.

Results

1. Experiments with isolated chloroplasts of *Elodea* leaf cells.

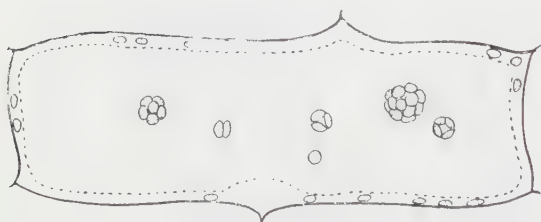


Fig. 2. A leaf cell of *Elodea densa* containing solitary chloroplast and chloroplast aggregates isolated into the vacuole by means of ultrasonic waves. $\times 540$

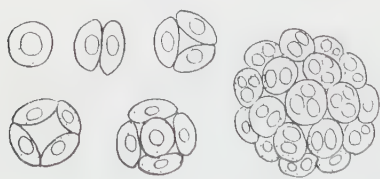


Fig. 3. Starch formation in *Elodea* chloroplasts ultrasonically isolated (after 16 days). $\times 1,000$

By appropriate treatment of *Elodea* leaf cells with ultrasonic waves, solitary chloroplasts, aggregates of two or more chloroplasts and cytoplasmic fragments with or without chloroplasts, all vibrating in Brownian motion, were brought into the vacuole (Fig. 2). Under the microscope, no solitary chloroplast was found to be accompanied by cytoplasm, whereas the chloroplast-aggregates appeared to have a minute amount of cytoplasm as cementing material and/or surrounding cytoplasmic film.

On further culture of the treated leaves on agar-plates or in aqueous media, starch formation in the isolated chloroplasts became more

* 'Solitary chloroplast' means a single chloroplast free from cytoplasm and nucleus.

** Mother cytoplasm means cytoplasm with a nucleus.

distinct, and the number of chloroplasts containing starch was statistically examined in both cases. Results were almost identical in two different cultures.

The solitary chloroplasts as well as the aggregates of two to seven chloroplasts retained green color in the vacuole during 16 to 24 day period (temperature: $20 \pm 2^\circ$), and appeared to be more active in starch formation in comparison with the chloroplasts embedded in the mother cytoplasm (Fig. 3). But, the former became gradually pale green towards the end of 16 to 24 day culture, resulting in alveolar or granular structure characterizing a necrotic symptom, and the latter became smaller and smaller in size and more and more yellowish in color, showing a symptom of degeneration.

On the other hand, no formation of starch grains took place in the chloroplasts accompanied by much cytoplasm. Such chloroplasts became gradually smaller in size and paler in green color. In several cases, it was observed that the cytoplasm had undergone swelling and autolysis due to ultrasonic damage, and that the chloroplasts were practically free from cytoplasm and vibrating in Brownian motion. They were small in size, green in color and varied in form, and never produced starch grain. Such forms of chloroplast resembled those which were previously obtained by Lubimenko²³⁾ on autolysis of tobacco leaf cells.

The results mentioned above are summarized in Table 1.

Table 1. Starch formation in chloroplasts isolated by ultrasonic treatment. Leaf cells of *Elodea densa* were exposed to ultrasonic waves and then cultivated for 6 days. Formation of starch in chloroplasts was detected by iodine-staining.

Chloroplast	Number of chloroplasts showing iodine reaction		Percentage of chloroplasts, showing positive iodine reaction
	Positive	Negative	
Solitary chloroplast	5	11	31
Aggregate of 2 chloroplasts	6	14	30
Aggregate of 3 chloroplasts	21	15	58
Aggregate of 4 chloroplasts	24	8	75
Aggregate of 5 chloroplasts	40	5	89
Chloroplast in the mother cytoplasm	0	30	0
Chloroplast in the intact cell	3	30	9
Total	99	113	..

As shown in Table 1, starch formation occurs in 31 % of the solitary chloroplasts observed and moreover, in chloroplast aggregate it is favored by an increased number of component chloroplasts. In contrast with this, no chloroplast in mother cytoplasm produced starch grain, whereas in an intact cell the starch formation was observed only in 9 % of the chloroplasts under the present culture conditions.

The starch grains formed in isolated chloroplasts increased gradually in size during 10 day culture in spring ($20 \pm 2^\circ$ from April to May), and during 23 days in winter ($7 \pm 2^\circ$ from Dec. to Feb.), and thereafter self-digestion took place before necrotic

symptom appeared in each chloroplast. The chloroplasts, no matter how isolated or in mother cytoplasm, remain alive during 10 to 24 days in spring and 40 to 55 days in winter.

2. Experiments with isolated fragments of *Spirogyra*-chloroplasts.

After treatment with ultrasonic waves, *Spirogyra* filaments were cultured on an agar-plate ($11\pm3^\circ$). In this experiment, several cells were viable for a long period of time (44 days and more), and even in some of the cells, in which chloroplast had been disrupted and some of its fragments dislocated into the vacuole, cell division could be observed.

The production and the following consumption of starch grains in isolated fragments of chloroplast were shown in Table 2 (cf. Fig. 4). It is seen from the table that the starch grains are formed in chloroplast fragementes more than 10 to 15 μ in diameter, but not in fragments less than 7 μ in diameter. Chloroplasts in mother cytoplasm or in an intact cell are capable of forming starch grains, the amount of which is, however, smaller than that in isolated fragments under the same experimental conditions. The starch grains once produced are gradually digested in the dark and disappear almost entirely after 24 to 42 days. When illuminated, starch

Table 2. Production and consumption of starch grains in isolated chloroplast fragments of *Spirogyra* in light or dark. (-: no starch, +: minute amount, ++: fairly large amount, +++: large amount, ++++: remarkably large amount of starch).

Date		March				April			Diameter of isolated chloroplast fragment (in μ)
		1	5	14	25	1	12	14	
Days after treatment with ultrasonic waves		0	4	13	24	31	42	44	
Conditions		dark	light	dark	"	"	"	light	
Cell sample A	Isolated chloroplast fragment	-	+++	+++	++	+	-	++	
	Chloroplast in mother cytoplasm	-	+	+	+	-	-	-	...
Cell sample B	Isolated chloroplast fragment (No. 1)	-	+++	++	+	-	10
	" (No. 2)	-	+++	++	+	-	10
	Chloroplast in mother cytoplasm	-	+	+	-	-
Cell sample C	Isolated chloroplast fragment (No. 1)	-	-	-	-	-	-	-	6
	" (No. 2)	-	-	-	-	-	-	-	7
	" (No. 3)	-	+++	+++	++	++	+	-	15
	" (No. 4)	-	+++	+++	++	+	+	+	14
	Chloroplast in mother cytoplasm	-	+	+	+	+	-	+	...

formation commences to take place in some isolated chloroplast fragments as well as in the chloroplast in mother cytoplasm.

Measured from the apparent size of starch grains produced, the activity of starch formation in the isolated fragments of chloroplasts appeared to be 8 to 27 times as great as that in the mother cytoplasm (Table 3).

It is noteworthy that, contrary to the case in *Elodea*, the chloroplasts of *Spirogyra*, whether isolated from or embedded in the mother cytoplasm, invariably remained green and active throughout the entire course of observation.

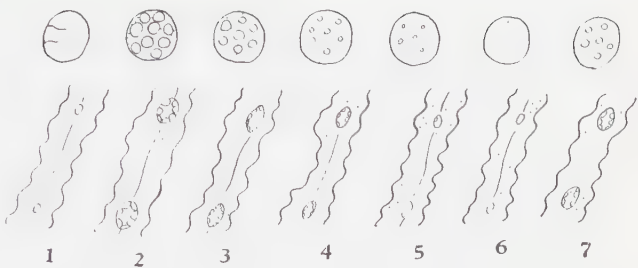


Fig. 4. The formation and consumption of starch grains in an ultrasonically isolated chloroplast fragment of *Spirogyra* (top), and an intact chloroplast band in the mother cytoplasm (bottom). Observed: (1) immediately after treatment, (2) after 4 days, (3) after 13 days, (4) after 24 days, (5) after 31 days, (6) after 42 days, (7) after 44 days. (1), (3)—(6): kept in the dark room, (2) and (7): stood inside the window under diffuse day light. $\times 430$

Table 3. Starch formation in fragments of *Spirogyra*-chloroplast isolated by ultrasonic treatment. On the 4th day culture, the volume of each starch grains was calculated from the average length of its diameter measured with the micrometer.

	Starch grains					
	Sample A		Sample B		Sample C	
	In isolated fragment of chloroplast (a)	In chloroplast with mother cytoplasm (b)	In isolated fragment of chloroplast (a)	In chloroplast with mother cytoplasm (b)	In isolated fragment of chloroplast (a)	In chloroplast with mother cytoplasm (b)
Diameter in μ (average of 10-23 starch grains)	3	1	2	1	3.5	1.2
Volume in μ^3	14.094	0.522	4.176	0.522	22.379	0.902
Ratio of average volume (a/b)	27.0		8.0		24.81	

Discussion

Recently, Arnon (1955) and his co-workers^{1),2),3),4)} succeeded in demonstrating the genuine photosynthetic function of chloroplast after its isolation into cell-free media by means of their grinding technique.* Similar results were also reported by Thomas

* Arnon *et al*^{1),2),3)} and Arnon⁴⁾ ground spinach leaves in 0.35 M NaCl or 0.5 M glucose under chilling, and separated whole chloroplasts by centrifugation at 1,000 *g* for 7 minutes; and after washing, the sediment was resuspended in NaCl or glucose solution. With this preparation they could demonstrate the whole process of photosynthesis.

et al.^{(29), (30), (31)} with the chloroplast fragments of *Spirogyra*. In search for a new isolation technique easily available under the microscope, the present author has devised the "intracellular micrurgical method" using ultrasonic vibration. This enables chloroplasts to translocate easily from cytoplasm into vacuole without disintegration of the cell wall.

By this method, chloroplasts of *Elodea* leaf cells were successfully brought into vacuoles in an isolated or aggregated state. With these isolated chloroplasts, starch formation was clearly demonstrated. The same fact was also ascertained with the chloroplast fragments of *Spirogyra* isolated by the ultrasonic waves. It is noteworthy that the chloroplasts free from both cytoplasm and nucleus appeared to be more active in starch formation than those having much cytoplasm containing no nucleus and than those in intact cells of a detached leaf. Such an accumulation of starch grains in isolated chloroplasts in the vacuole is, of course, due to the maintenance of an active starch formation in individual chloroplasts on the one hand, and is also to be accounted for by the assumption that the isolated chloroplasts are free from mother cytoplasm on the other, which may consume or transform the accumulated starch grains into an invisible state.

Now, our attention should be drawn to the following two points: the one is whether the solitary chloroplasts showing the ability of starch formation are practically accompanied by no cytoplasmic layer, and the other is whether the isolated chloroplasts devoid of photosynthetic ability are damaged in their submicroscopic structures, in their permeability and/or in their physico-chemical properties.

As regards the first problem, it is uncertain whether the isolated chloroplasts are completely free from cytoplasm, in so far as the microscopic observations are concerned. Even if the isolated chloroplasts were covered with submicroscopic film of cytoplasm, it is not conceivable that such a minute amount of cytoplasm would be essential for the photosynthetic process, except for the maintenance of surface structure or semipermeability of the isolated chloroplasts. In this connection, it should be remembered that an excess of cytoplasm was found rather to be unfavorable for starch formation.

Concerning the second problem, it appears that drastic manipulations used by many workers for the isolation of the chloroplasts, e. g. squeezing or grinding of the cells, may possibly change the fine structure (physico-chemical state) of the intact chloroplasts, resulting in a loss of photosynthetic activity. This view may well be supported by our observation that the permeability of the chloroplast appreciably increases after such drastic treatment (unpublished).

As mentioned above, Arnon⁽⁴⁾, and Thomas *et al.*^{(9), (30), (31)} have, however, shown that the chloroplasts isolated by their grinding method could evoke photosynthesis indeed. This finding is of great importance, in view of a general concept that the isolated chloroplasts are capable of splitting water molecule photochemically, but incapable of fixing carbon dioxide for the purpose of starch formation. Their statements

are in good accord with our findings on the chloroplasts isolated by ultrasonic method in relation to the photosynthetic activity.

Summary

1. By an appropriate treatment with ultrasonic waves, the chloroplasts of *Elodea densa* and the fragments of chloroplast band of *Spirogyra* were liberated from the cytoplasm and brought into the vacuole within the same cell. In these isolated chloroplasts the formation of starch was demonstrated photosynthetically.

2. The amount of starch formed in the isolated chloroplasts which were free from cytoplasm and nucleus was larger than that formed in the chloroplasts embedded in cytoplasm with or without the nucleus or in intact cells of a detached leaf.

3. The isolated chloroplasts remained alive so long as 55 days in the vacuole of the mother cell. The starch grains once formed in the isolated chloroplasts were consumed gradually in the course of cultures in light or dark conditions.

4. The isolated chloroplasts of *Elodea* remained green in color, indicating their normal state, while those in the mother cytoplasm became smaller in size and more yellowish in color, showing a symptom of degeneration. However, in *Spirogyra*, the isolated chloroplast fragments with or without mother cytoplasm remained green in color through the entire course of experimentation.

5. It was discussed whether the isolated chloroplasts having the ability of starch formation were entirely free from cytoplasmic layer, and whether the isolated chloroplasts deprived of photosynthetic ability would suffer from any damages in their submicroscopic structures or in their physico-chemical properties.

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Addendum

After this paper was submitted to the press, the author has found the publication of A. Moyse in which he reported that "isolated chloroplasts possess an enzymatic equipment, which is capable of performing all steps of photosynthesis in the presence of light. This is possible *in vitro* in the absence of living cells, whereas complete chloroplasts are required." (*Année Biol.* 32: 5 (1956); *Biol. Abst.* 31: 29543 (1957)).

摘 要

生細胞をすりつぶし法などで破壊して葉緑体を細胞外にとり出すと光合成は行われなくなるが、最近 Arnon ら(1954, 1955) は同じすりつぶし法で遊離したホウレンソウの葉緑体が完全な光合成系であることを唱え、続いて Thomas ら (1955, 1957) はアオミドロの遊離葉緑体でも生細胞でと同程度の光合成を行うことを報じた。

私は 1949 年にオオカナダモやアオミドロの細胞を用いて、超音波作用で葉緑体や葉緑体片を液胞内に遊離させ、それらが光でデンプン形成を行うことを予報した。

ここではその後に得た実験結果をも加えて、これらの遊離葉緑体の光合成によるデンプン形成について、より詳しく報じた。その大要は次のごとくである。

1. 適当な強さの超音波作用でオオカナダモの葉の葉緑体や、アオミドロの葉緑体片を液胞内に遊離させることができる。
2. これらの遊離葉緑体は細胞質や核を伴う葉緑体の場合よりも多量のデンプンを形成する。
3. 遊離葉緑体は生きた細胞の液胞内で 55 日間も生きている。
4. オオカナダモの遊離葉緑体は正常な緑色であり、その母原形質内の葉緑体はしだいに小さく、また黄化し、退化の徴候を示すが、アオミドロでは遊離葉緑体片だけでなく母原形質内の葉緑体も緑色のままである。
5. デンプン形成能をもつ遊離葉緑体が真に細胞質を伴っていないかどうか、また光合成能がないとされた遊離葉緑体は、遊離に際してその微細構造に物理化学的変化をうけなかったかどうかについて論議した。

Studies on the Nucleus of the Spore in *Streptomyces*

Part I. Observations on the Nuclear Substance of the Spore in *Streptomyces* which belong to A Group

by Shukuo KINOSHITA* and Shiro ITAGAKI*

木下祝郎*・板垣史郎*: 放線菌胞子の核に関する研究 第1報 A 群に属する放線菌の
胞子の核物質の観察

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Reviews^{1), 2), 3), 4), 5)} and reports concerning the nuclei of microorganisms are not rare. Recently, observations on the nuclei of various species of microorganisms were published and even the mitosis^{6), 7), 8), 9), 10)} was observed on some species.

There are some nuclear staining techniques according to which the nuclear substances are stained with the basic dyes after removal of basophilic cytoplasm by suitable treatments. Though there are many discussions regarding the nuclear staining, even on the Feulgen reaction, authors, however, do not touch on this problem in the present study.

So-called Robinow's hydrochloric acid-Giemsa method (Robinow's method) which was introduced by Piekarsky¹¹⁾ and developed by Robinow⁴⁾ has been generally used by many workers since this method is very simple and good for obtaining clear preparations within a short times.

Plöth¹²⁾ studied some strains of Actinomycetaceae (A, B, C, D, G, St, Sm, M, J, and My) isolated from the forest, desert land, old coagulated milk and nodule, and published clear microphotographs of the nuclear substance of spores in some strains. Carvajal¹³⁾ studied on *S. griseus* by the aid of electron microscope, and reported the spores were uninucleate or multinucleate.

Klieneberger-Nobel¹⁴⁾ stained the nucleus of *A. gardneri* N. C. T. C. 6531, *A. albosporus* N. C. T. C. 1578, *A. chromogenes* N. C. T. C. 1569 and *A. madurae* N. C. T. C. 1070, and studied on the life cycle, germination and sporulation-process of *Actinomyces*. He described that the nuclear cylinder of the secondary hyphae was divided into a pair of chromosomes which change into the nucleus of the spore. Webb, Clark and Chance¹⁵⁾ stained the nucleus of *Nocardia corallina* (ATCC-4273), *N. blackwelli* (ATCC-6846) and *S. griseus* (ATCC-10137). They applied Chance's method¹⁶⁾ and examined the conditions of the nuclear staining. It was shown that the concentra-

* Tokyo Research Laboratory, The Kyowa Fermentation Industry Co., Ltd, Tokyo, Japan.
協和醸酵工業株式会社東京研究所

tion of nigrosin for good decolorization was 5 % for *N. corallina* and *S. griseus*, and 1 % for *N. blackwelli*. Hagedorn¹⁷⁾ observed nuclei in the spores, germ tube and mycelium of *S. aureofaciens* with the phasecontrast microscope, and studied on the influences of the several neutral inorganic salts.

In the present study the writers studied on the number and shape of nuclei of the spores in *Streptomyces*.

The conditions of the staining of the spores were examined on *S. lavendulae* at first, then observed on the nuclei in the spores of *Streptomyces* which belong to A group according to seventh edition of Bergey's "Manual of Determinative Bacteriology"¹⁸⁾.

Methods and Results

1. Determination of the conditions of the nuclear staining of the spores of *Streptomyces*.

S. lavendulae ATCC-8664 was used for the experiment. It was cultivated on Bennet's agar slant at 28° for about two weeks. Robinow's method was used for the fixation, hydrolysis and Giemsa's staining.

A small block of agar on which the culture was heavily sporulated was cut off and impression preparations were prepared. These preparations were dried and fixed with osmium tetroxide vapor (2 % solution).

After fixation, it was washed with tap water and hydrolyzed with 1N-hydrochloric acid. After washing, it was stained with Giemsa's solution and then examined with a microscope.

1) Time of fixation.

Time of fixation using osmium tetroxide vapor was one, five, ten, thirty or sixty minutes. The material was hydrolyzed with 1N-hydrochloric acid for ten minutes at 57-58° and then stained with Giemsa (1:20) solution for thirty minutes.

Nuclei were stained well in all cases and no significant difference of results was observed within these time ranges.

2) Temperature and time of hydrolysis with 1N-hydrochloric acid.

Time of hydrolysis was two, five, ten, thirty or sixty minutes. The temperature was 40°, 50° or 60°. The material was fixed for 1.5 minutes and stained with Giemsa for thirty minutes.

At 40°, hydrolysis within two to ten minutes was insufficient, nucleus and spore-plasm were not discerned by two to five minutes hydrolysis; good results were obtained by thirty to sixty minutes hydrolysis.

At 50°, with two minutes hydrolysis nucleus was not discerned at all. With ten to thirty minutes hydrolysis it was stained clearly. With sixty minutes hydrolysis nucleus was not found at all and spores were stained lightly.

At 60°, with five to ten minutes hydrolysis the nucleus stained clearly. Thirty to sixty minutes hydrolysis nucleus was not discerned.

3) Concentrations of Giemsa's solution and the time of staining.

One part of Giemsa original solution was mixed with five to forty parts of dist. water.

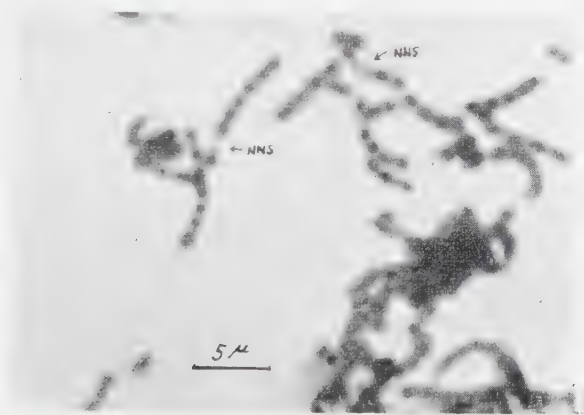
Other conditions were as follows: The vapor of the 2 % osmium tetroxide solution was used to fix the material for one to 1.5 minutes. The hydrolysis was done with 1N-hydrochloric acid for ten minutes at 50°.

There was no difference with these concentrations and time ranges in results.

2. Observations on the nuclear substances of the spores in various species of *Streptomyces* which belong to A group.

A group according to Bergey's classification does not produce the soluble pigments on the organic media.

1) *S. albogriseolus* NRRL-B-1305 (Microphoto. 1)



Microphoto. 1. *S. albogriseolus* NRRL B 1305. There were a few rod-like and dumbbell shaped nuclei. None-nucleate spores exist rarely.

NNS : none-nucleate spore

One nucleus was observed in each spore which was irregular in size. Usual form of the nucleus is spherical or ellipsoidal, but there are a few rod-like and dumbbell forms. Dumbbell shaped nucleus has been reported in *E. coli* and *B. megatherium*. It has been located at right angle against at long axis of the cell, but the dumbbell shaped nucleus of the spore in *S. albogriseolus* NRRL-B-1305 is situated along the long axis, according to our observation.

Spores which have two nuclei were rare.

2) *S. sp.* NRRL-2288

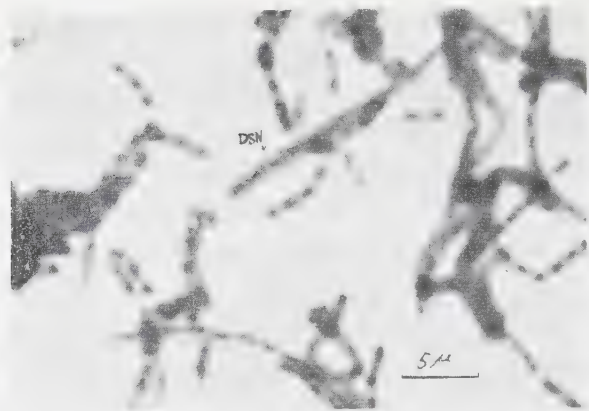
Uninucleate at all.

3) *S. coelicolor* 9023 (Microphoto. 2)

Most of the spores of this strain are uninucleate, but rod-like and dumbbell shaped nuclei are not so rare.

The existence of two nuclei in a spore is obvious, but three nuclei are not seen at all.





These distributions were shown in Table 1.



Microphoto. 2. *S. coelicolor* 9023. Spores are irregular in size. Various types of nuclei are shown, rod-like and dumbbell shaped.

DSN : dumbbell shaped nucleus

Table 1. Distribution of the shapes of nuclei in *S. coelicolor* 9023

Shape of nucleus	Distribution %
	87
	7
	8
	5
others	2

4) *S. californicus* ATCC-3312

Uninucleate at all.

5) *S. flaveolus* P-10

Uninucleate at all.

6) *S. rimosus* NRRL-2234

Nucleus is observed, but the spore coat and spore plasma are not observed separately.

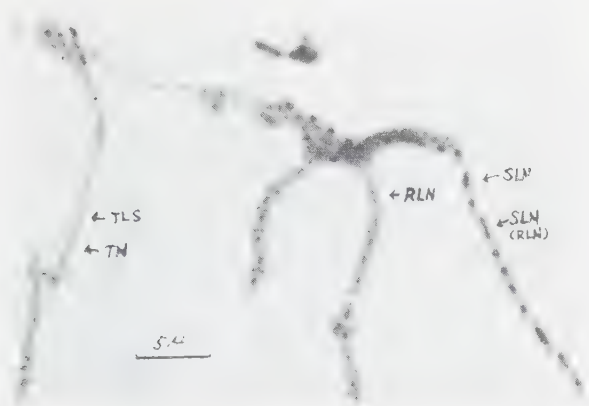
7) *S. griseoflavus* #305 (Microphoto. 3)

Nuclear substance is stained clearly. There are various types of nuclei; namely, dumbbell shaped and two nuclei. The shape of the spore which has a dumbbell shaped nucleus or two nuclei was longer than usual spore.

These distributions are shown in Table 2.

The dumbbell shaped nucleus of the spore in *S. griseoflavus* # 305 differs from that of *S. coelicolor* 9023. Two nuclei are jointed with a thread-like structure in the former. This thread-like structure may have a special role in physiological function of the spore.

Specially large nuclei were observed rarely.



Microphoto. 3. *S. griseoflavus* #305. Dumbbell shaped nucleus is thought to be that two nuclei, jointed each other with a thread-like structure. Specially large nuclei are observed.

RLN : rod-like nucleus SLN : specially large nucleus
TN : two nuclei TLS : thread-like structure

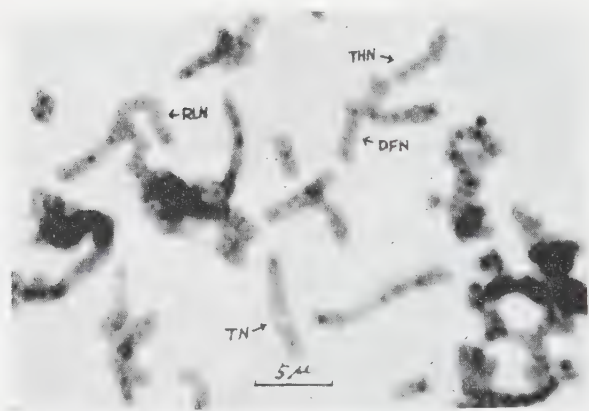
Table 2. Distribution of the shapes of nuclei in *S. griseoflavus* #305

Shape of nucleus	Distribution %
	87
	9
	2
	2

8) *S. flavovirens* ATCC-3320
Uninucleate at all.
9) *S. griseoluteus* P-37 (Microphoto. 4)
Two nuclei are comparatively many, rod-like and dumbbell shape are rarely observed.
The cases of three nuclei are very rare, but the nuclei are observed clearly.
These distributions are shown in Table 3.

Table 3. Distribution of the shapes of nuclei in *S. griseoluteus* P-37.

Shape of nucleus	Distribution %
	92
	0.5
	0.5
	6
	1



Microphoto. 4. *S. griseoluleus* P-37. Various types of nuclei are shown. Three nuclei are very rare, but observed clearly.

RLN : rod-like nucleus TN : two nuclei
DFN : dumbbell shaped nucleus THN: three nuclei

The distributions of the number and shape of nuclei in the spores of *Streptomyces* which belong to A group are shown in Table 4. When counted, rod-like nucleus was counted as uninucleate.

Dumbbell shape nuclei are counted as two nuclei.

Table 4. Distributions of the number and shape of nuclei in the spores of *Streptomyces* which belong to A group.

Strains	number %			Shape %				
	1	2	3					others
<i>S. albogriseolus</i> NRRL-B-1305	100	rare	—	100	rare	rare	rare	—
<i>S. sp.</i> NRRL-2288	100	—	—	100	—	—	—	—
<i>S. coelicolor</i> 9023	85	13	—	78	7	8	5	2
<i>S. californicus</i> ATCC-3312	100	—	—	100	—	—	—	—
<i>S. flaveolus</i> P-10	100	—	—	100	—	—	—	—
<i>S. rimosus</i> NRRL-2234	100	—	—	100	—	—	—	—
<i>S. griseoflavus</i> #305	96	4	—	87	9	2	2	—
<i>S. flavovirens</i> ATCC-3320	100	—	—	100	—	—	—	large nucleus
<i>S. griseoluleus</i> P-37	92.5	6.5	1	92	0.5	0.5	6	—

Discussion

Nine strains which belong to A group of *Streptomyces* were nuclear stained and observed. Following six strains were uninucleate at all: *S. sp.* NRRL-2288, *S. cali-*

formicus ATCC-3312, *S. flaveolus* P-10, *S. rimosus* NRRL-2234, *S. flavovirens* ATCC-3320 and *S. albogriseolus* NRRL-B-1305.

S. albogriseolus NRRL-B-1305 seems to be uninucleate, but the existence of two nuclei was recognized.

Two nuclei were recognized more than 10 % spores of *S. coelicolor* 9023. It is very remarkable that three nuclei have been observed in *S. griseoluteus* P-37 obviously. The distributions of the number and shape of the nucleus are shown in Table 4., but the percentages are not so strict, because these percentages have been examined by counting of the nuclei of several hundreds spores upon which clear observation has been possible.

The shape of nucleus is spherical or ellipsoidal, and nucleus is situated at the center of spore.

In general, the spore which has two nuclei is larger than usual one.

When two nuclei come in contact each other, these may be observed as a dumbbell shape. At first glance, it seems that dumbbell shape is due to incomplete observation by microscope. It is observed, however, in *S. griseoflavus* #305 that a thread-like structure is connecting two separated nuclei in a spore. This fact may show that dumbbell shape is not merely formed by the contact of two nuclei. The meaning of the rod-like nucleus should be considered carefully. Beutner⁹⁾ reported rod-like nucleus in the stage of mitosis in a cell of *B. megatherium*. He called it the axial chromatin. The present writers can not understand the rod-like nucleus of the spore of *S. griseoflavus* #305 as the same figure as the axial chromatin of *B. megatherium*.

Summary

In this paper, nuclear substance of the spore in *Streptomyces* was studied in connection with the general cytological studies.

The conditions of the nuclear staining of the spores were examined and observed on the nucleus in nine strains of *Streptomyces* which belong to A group. The results were as follows:

1) Six strains out of the nine most spores were uninucleate. In this case, the shape of nucleus was generally spherical or ellipsoidal.

2) In four strains out of nine, namely *S. coelicolor* 9023 (13 %), *S. griseoluteus* P-37 (6.5 %), *S. griseoflavus* #305 (4 %) and *S. albogriseolus* NRRL-B-1305 (rare), two nuclei were observed in one spore.

3) Three nuclei were rarely observed in the spores of *S. griseoluteus* P-37. There were no strains which have more than four nuclei in one spore.

4) In some spores of *S. coelicolor* 9023, *S. griseoflavus* #305, *S. griseoluteus* P-37 and *S. albogriseolus* NRRL-B-1305 the rod-like or dumbbell shaped nucleus was observed.

5) Abnormally large nuclei were observed in the spores of *S. flavovirens* ATCC-3320.

The writers wish to express their sincere thanks to Prof. A. Yuasa and Mr. H. Saito of the University of Tokyo for their kind advice and suggestion. Thanks are also due to Mr. M. Furukawa for his assistance.

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摘 要

一般的な細胞学的研究に関連して、放線菌孢子の核物質の観察を行い、A群に属する9株につき報告した。

(1) 6株はほぼ完全に単核である。核の形は、一般に球形または楕円形である。

(2) 4株、すなわち *S. coelicolor* 9023 (13%), *S. griseoluteus* P-37 (6.5%), *S. griseoflavus* #305 (4%) および *S. albogriseolus* NRRL-B-1305 (稀) において2核を認めた。

(3) *S. griseoluteus* P-37 において、きわめてまれながら、明らかに核を認めた。4核以上の多核孢子は9株中いずれにも認められなかった。

(4) *S. coelicolor* 9023, *S. griseoflavus* #305, *S. griseoluteus* P-37 および *S. albogriseolus* NRRL-B-1305 の4株において、桿状あるいは垂鈴形核を認めた。

(5) *S. flavovirens* ATCC-3320 において、異常大型核を認めた。

Developmental Mechanics of Fucaceous Algae XI. Liberation of Small Bodies in *Coccophora* Eggs*

by Singo NAKAZAWA**

中沢信吾：フーカス科藻類の発生力学 XI. コギモクの卵に存在する小体の放出

Received August 22, 1958

In the oogenesis of Fucaceous algae, the diploid mother nucleus of the oogonium undergoes three successive divisions resulting in eight haploid nuclei. Then, in *Fucus*, the contents of the oogonium are divided into eight equal parts and each appropriates one nucleus, so that eight oospheres are born from the same oogonium⁴⁾. In *Carpophyllum flexuosum*¹⁾, *Cystophyllum cismyrioides*¹⁷⁾, *Hizikia*¹⁸⁾, *Sargassum*¹⁷⁾, and in *Turbinaria*⁵⁾, different from *Fucus*, the contents of the oogonium are not divided, so that but one oosphere, containing eight nuclei, is born. There are several types between these extremities. In *Hormosira*³⁾, for instance, four oospheres develop in the same oogonium, each containing one nucleus, and the other four nuclei are cast out of the oospheres, there they are degenerated. In *Cystoseira*^{2), 14)}, only one oosphere containing one large nucleus develops in each oogonium, casting out the seven superfluous nuclei. As to the oogenesis of *Coccophora*, some doubts, regarding the number of oospheres, were expressed by Oltmanns¹⁵⁾ in 1889. Afterwards, it was studied by Smith¹⁶⁾ and was found to develop but one oosphere, as Oltmanns presumed. Further, it was observed by Tahara¹⁸⁾ that *Coccophora* oosphere came to appropriate only one nucleus as a result of disintegrating seven out of eight nuclei in the oospheric cytoplasm. Sometimes, therefore, there develop abnormally matured oospheres containing eight nuclei caused by non-occurrence of the nuclear disintegration just as in *Sargassum*¹¹⁾. However, the writer has noticed that there are one to several small bodies in the perioospheric space of *Coccophora* eggs looking similar to the eliminated nuclei in the egg of *Cystoseira Osmundacea* illustrated by Gardner²⁾. Their origin, revealed by this experiment, seems to present a further evidence indicating a change of the egg membrane after the fertilization, a fact which was remarked previously^{9) 12) 13)}. This is the subject-matter of this paper.

On April 8th, 1958, female branches of *Coccophora Langsdorfii* were collected and cultured at the Marine Biological Station of Asamushi. Before their cultivation, the branches were washed with boiled sea water five times repeatedly to remove possible spermatozooids attached in the marine. Boiled sea water was used for the culture throughout the experiment. The medium was renewed twice a day. By

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**Biology Department, Yamagata University, Yamagata, Japan. 山形大学生物学教室

this means, the receptacle was placed under a sterilized condition, i.e., the egg discharge was expected in a completely sperm-free environment. On the other hand, male receptacles were picked off, washed, then arranged on filter paper sheeted in Petri dishes and wetted with boiled sea water, and covered to retain moisture for getting sperm. On 12th April, both the egg and the sperm were discharged. Artificial insemination was carried out. Eggs before and after fertilization were observed. Brilliant green, Congo red, Janus green B, methylene blue, neutral red, and Nile blue were tested for staining the small body. Tannic acid-iron method was applied to reveal the structure of the small body and it was successful. Aceto-carmin and alcohol solution of Sudan III were also tested.

It was affirmed that there were one to several small bodies around the oosphere (Fig.1). It is true that their number differs according to the egg, although it is

difficult to determine the accurate number because some of the small bodies cannot be observed as they are concealed on the other side of the oosphere. That is, their locality is not definite. The small body is a colorless sphere, about 7μ in diameter. It remains beside the oosphere throughout the progress of cleavage, and finally is disintegrated before completion of the embryo. It shines under microscope refracting light. The small bodies cannot be gathered even if the egg is centrifuged at 1500 times gravity for 20 minutes, that is, they are almost of the same specific gravity as that of the medium filling the oospheric space. It had been thought that the small bodies were spermatozooids degenerating around the oosphere having failed in fertilization. This,

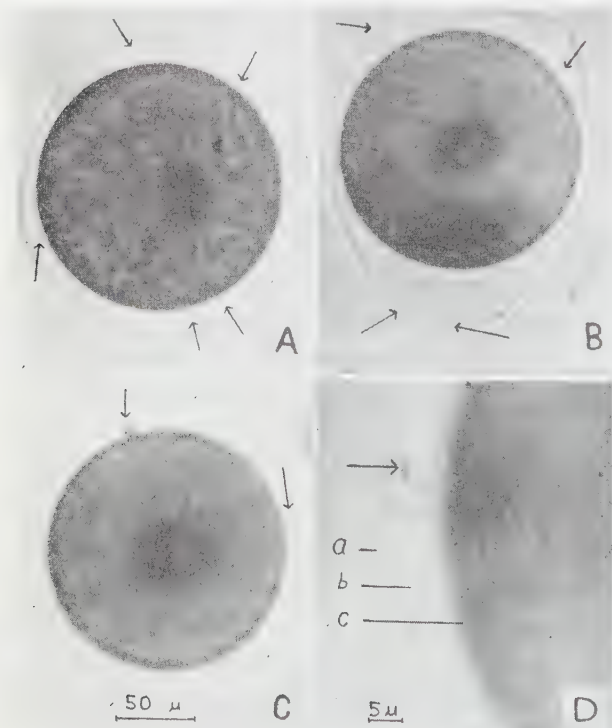


Fig.1. A, B) Small bodies around the oosphere, C) the same stained with Congo red, D) enlargement of a small body. a, inner wall of the mucilaginous coat; b, perioospheric space; c, oosphere. Small bodies are indicated with arrows.

however, was rejected as it became clear that the small bodies were also observed before the insemination. Cutting receptacle, still undischarged eggs were inspected, but there the small body was not perceived. This indicates that the small body appears after the egg is discharged out of the receptacle. However, it is noteworthy

that the small body is not observed in the egg discharged artificially by Nakazawa's method¹⁰⁾, i.e., by means of setting receptacle in a hypotonic sea water diluted to 1/5 in density.

The origin of the small body was revealed by observation. At first, a clear small protuberance arises at a part on the surface of the oosphere (Fig. 2). The protuberance grows outwards gradually, and finally is liberated as a small body. In the unfertilized egg, the liberation takes place successively at various parts on the surface until several small bodies are counted. If fertilization occurs before or amid the liberation of small bodies, the liberation ceases after an hour or so, though only one or two, or sometimes none, have been liberated. The small body is stained blue with Nile blue, red with Congo red, but is not stainable with brilliant green, eosin, Janus green B, methylene blue, neutral red, and with alcohol solution of Sudan III. The central part of the small body is stained deep red with aceto-carmine, revealing a chemical differentiation into the central and the peripheral substances. By immersing eggs into 0.1 per cent ferric chloride solution after they are soaked in 0.1 per cent tannic acid for ten hours and then rinsed for ten minutes, the small body was stained black revealing the same structure as that observed with aceto-carmine. The small body could not be dissolved with 70 per cent alcohol.

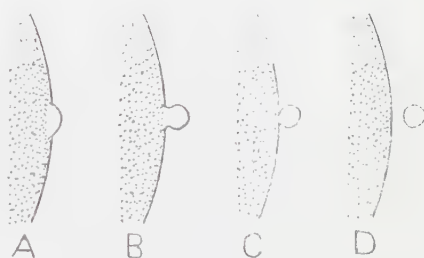


Fig. 2. Liberation of small body

The staining with aceto-carmine definitely implies that the small body contains chromatin. Therefore, it seems that they are superfluous haploid nuclei eliminated from the mature oosphere. However, judging from the fact that the number of the small body differs according to the egg, it seems that not always all of the seven nuclei are body eliminated, but some are disintegrated in the egg cytoplasm. The latter, probably, led Tahara¹⁸⁾ to elaborate the idea that the superfluous nuclei were all degenerated in the cytoplasm. Further investigations, especially as to the intracellular origin of the small body, are required to confirm the present interpretation. The writer's interests are directed rather to the fact that the liberation of small body ceases after fertilization. Because this seems to be another fact in support of the argument that the nature of the egg membrane has changed after fertilization. The egg membrane is very soft and no or very little cellulose can be detected there, but it becomes thicker and cellulose is deposited gradually after fertilization as is known in *Fucus*⁶⁾, *Hormosira*⁷⁾, *Coccophora* and in *Sargassum*^{12, 13)}. Owing to this change, especially in *Coccophora*, the egg form is stabilized, and this results in polarity determination¹³⁾. Making use of this change Whitaker¹⁹⁾ caused *Fucus* eggs be elongated artificially, sucking them into a capillary having a smaller diameter before deposition of cellulose; after the egg form is stabilized in the capillary by deposition of that substance, they are

jetted out. However, the cellulose deposition seems to begin at least five or more hours after fertilization in *Coccophora*, and the egg membrane is still so soft about an hour after fertilization that a morphogenetic movement begins to take place. Therefore, some other change in the property of the membrane must be responsible for the ceasing of the liberation of the small body, which occurs about an hour after fertilization. On the other hand, it is known that the oil cap is extruded out of the oosphere when the egg is strongly centrifuged before fertilization, while this does not occur 20 minutes after fertilization⁹. Similar change was also reported in *Fucus*⁸. Thus, the ceasing of the liberation of small bodies seems to be caused by a change of this kind in membrane properties, a change which occurs within a short lapse of time after fertilization.

Summary

(1) Small clear protuberances grow and then are liberated from several parts on the surface of the unfertilized oosphere of *Coccophora Langsdorfii*. The number of the small bodies liberated was one to several. The central part of the small body can be stained with aceto-carmin, implying that the small body consists of nuclear substances.

(2) The liberation of small bodies is considered to be the partial or total elimination of the superfluous haploid nuclei, which were born through the meiosis of oogonium. They are seven in number theoretically.

(3) The liberation ceases about an hour after fertilization. This indicates that a change is taking place in the nature of the egg membrane after fertilization.

The writer's sincere thanks are due to Emer. Prof. Masato Tahara of the Tohoku University and to Prof. Shumpei Inoh of the Okayama University for their valuable suggestions.

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摘 要

(1) スギモク *Coccophora Langsdorfii* では未受精卵の表面の数カ所において透明な小突起を生じ、それらは開卵腔に放出される。放出された小体の数は1個から数個におよび、小体の中央はアセトカーミンで染色される。これは小体が核物質をもつことを示す。

(2) 小体の放出は蔵卵器の減数分裂によって生じた8個のハプロイド核のうち不用の核(理論上7個)の全部あるいは一部が排除される現象とみられる。

(3) 受精後約1時間たつと小体の放出はおこななくなる。これは受精によって卵膜の性質に変化がおこっていることをあらわす。

Rearrangement of the Systematic Position of a Thermal Alga, *Cyanidium caldarium**

by Hiroyuki HIROSE**

広瀬弘幸**: 温泉産イデユコゴメの分類学的位置の再変更

Received July 11, 1958

The first discoverer of the present alga is J. E. Tilden¹⁴⁾ who found this alga in Yellowstone Park and she reported this alga in 1898 as a green alga and gave a new name, *Protococcus botryoides* forma *caldaria*. Next in 1901, believing the plant to be a blue-green alga, W. A. Setchell¹³⁾ made a new combination, *Pleurocapsa caldaria* (Tilden) Setchell. Studying the present alga from Malay, G. S. West¹⁵⁾ described this alga in 1904 as a new member of green algae, *Palmellococcus thermalis* nov. sp., but he referred neither to Tilden's new forma nor Setchell's new combination. Later in 1933 and 1936 L. Geitler^{7), 8)} studied the present alga which was found in Sunda Islands and concluded that it was quite a unique species which not only belonged to a new genus, but was ranked as a new family and established the Cyanidiaceae with *Cyanidium caldarium* (Tilden) Geitler. In the same year (1936) J. J. Copeland²⁾, studying the present alga found in Yellowstone Park, established a new genus *Pluto* and named *Pluto caldarius* (Tilden) Copeland, but never referred to the Geitler's new name.

Many Japanese scientists such as Y. Okada¹²⁾, Y. Emoto^{4), 5)}, Y. Yoneda⁵⁾, H. Hirose⁴⁾, and K. Negoro¹¹⁾ adopted *Cyanidium caldarium* (Tilden) Geitler as the species name of the present alga. In 1950 H. Hirose¹⁰⁾ emphasized that the present alga had to be rearranged to a member of green algae belonging to the genus *Chlorella* and proposed a new name, *Chlorella caldaria* (Tilden) Hirose. A little later in 1954 M. B. Allen¹⁾ made just the same point of an argument as Hirose's and proposed a new name, *Chlorella caldaria* (Tilden) Allen.

Considerations based on the results of the present study lead to a conclusion that the systematic position of the present alga comes much nearer between *Porphyridium* and *Rhodospira* of red algae. Consequently the author wishes to establish a new generic and specific name, *Rhodococcus caldarius* (Tilden) Hirose comb. nov.

Materials and Methods

Materials which were used in the present studies were collected by the author

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** Department of Botany, Faculty of Science, Kobe University, Kobe, Japan. 神戸大学理学部植物学教室

at Jigokudani valley of Noboribetsu spa in Hokkaido besides those collected by Mr. Y. Tsubo at Washikura spa in Fukushima Prefecture. These materials were cultured at our laboratory. Experiments were carried out on these cultured materials. In order to ascertain existence of phycocyan, Abbe's microspectroscope and Beckman's spectrophotometer were used. Feulgen reaction method was utilized for an observation of chromatins. To examine properties of assimilatory product, the algal cell was treated with jod-jod kali, jod-chlorhydrate and chlorzinkjodine.

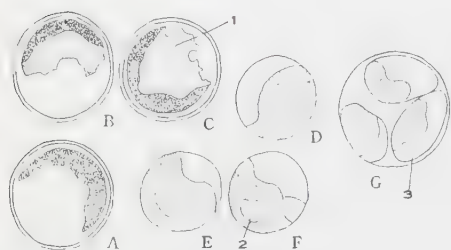


Fig. 1. Structure of cells. A, B, C. Matured individual cell: definite cell wall and plastid are shown. \times ca. 2500. D, E, F. Juvenile individual cell; E and F showing perforation of plastid. \times ca. 2500. G. sporangium which contains four endospores, three of which are drawn. \times ca. 2500. 1. chloroplast. 2. perforation. 3. endospore. All figures are redrawn from H. Hirose (1950).

but shows quite similar properties to those of phycocyan of a blue-green alga, *Phormidium corium* shown in Fig. 2 C.

Comparing the absorption curve of the present alga with that of Chlorophylls extracted from *Chlorella pyrenoidosa* (Fig. 3) through Beckman's spectrophotometer, two curves in the

figure 3 show that there exists little difference between the peak I and the peak II of the present alga despite the fact that much difference can be seen between the peak I and the peak II of *Chlorella pyrenoidosa*. This fact can not be explained without assuming that there exists one more element of a strong absorption besides

Observations and Experiments

A. Morphology of a cell: Shape of a living cell is spherical and contains a parietal laminate chloroplast which lacks pyrenoid. (Fig. 1 A-G).

B. Phycocyan: As shown in text-figure 2 E, an absorption spectrum of cell aggregations through microspectroscope shows that the strongest absorptions are located respectively at a little left-side of $700m\mu$ and at a little right-side of $620m\mu$, and one more strongly absorbed zone between $450m\mu$ - $520m\mu$. The spectrum never coincides with that of *Porphyridium cruentum* (Fig. 2 B),

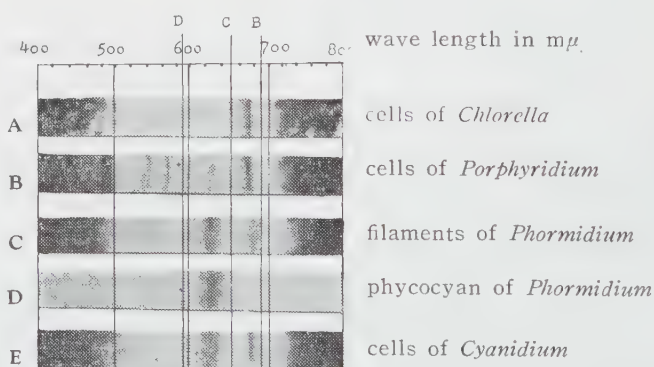


Fig. 2. Absorption spectra of living cells of *Cyanidium caldarium*, *Chlorella pyrenoidosa*, *Porphyridium cruentum*, *Phormidium corium*, and water extract of phycocyan of *Phormidium*.

chlorophyll *a* in a *Cyanidium* cell.

The above two experiments lead to the conclusion that there exists phycocyan within a cell of the present alga as M. B. Allen¹⁾ described in 1954.

C. Chlorophylls: The present alga contains merely chlorophyll *a* and lacks chlorophyll *b*, as was already clarified by H. Hirose¹⁰⁾ (1950) and M. B. Allen¹⁾ (1954).

D. Nucleus: A mode of distribution of chromatin within a cell is shown in Fig. 4 A-L. Densely aggregated chromatin that are always

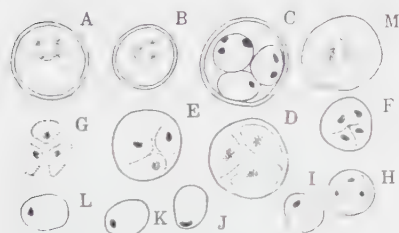


Fig. 4. Distribution of chromatin within a cell. A, B. Nucleus-like structure. C-H. Endospore formation. C. A mother cell containing three endospores which contain two to three aggregations of chromatin. I-L. One of endospores each of which respectively contains small but condensed aggregation of chromatin. All figures \times ca. 1900.

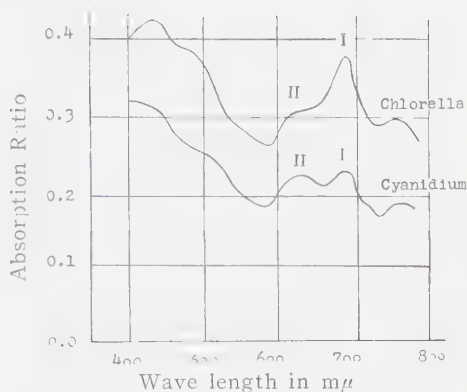


Fig. 3. Curves showing the distribution of light absorption of total pigments in living cells of *Chlorella* and *Cyanidium*.

definitely out-lined are observed within a cell. This definitely shaped mass of chromatin has better be called a nucleus which comes nearer to those of Rhodophyceae or other higher algae, rather than be called centroplast of Cyanophyceae or those of Schizophyceae.

E. Assimilatory product: An identification of polysaccharide as an assimilatory product is not completed. However, through an observation of colour-tone-changes of cell contents when treated with iod-reagents, the assimilatory product of the present alga comes close to floridean starch or glycogen. Cell-contents are stained in red, wine-red, or brown-red.

Discussion

The characteristics of the present alga are newly clarified as follows from the above-mentioned observations and experiments. (1) The plant body is unicellular and spherical. (2) Plant is reproduced merely by means of endospore-formation. (3) The plant cell has a definitely shaped chloroplast, which is single, and parietal laminate, without pyrenoid. (4) The plant cell contains only chlorophyll *a* and never has chlorophyll *b*. (5) Phycoerythrin is not contained, but merely phycocyan exists as a sort of phycobilin. (6) The plant never produces starch as an assimilatory product, but a kind of soluble polysaccharide which comes nearer to floridean starch or dextrine. (7) Chromatins aggregate to form a mass which may be called

nucleus. When the systematic position of the present alga is considered based on the above-mentioned seven characteristics, the following conclusions are reached. Firstly being based on the characteristics No. 5 (existence of phycocyan) and No. 6 (nullity of starch), the alga must be transferred from Chlorophyceae to either Cyanophyceae or Rhodophyceae. Lastly being based on the characteristics No. 3 (existence of a definitely shaped chloroplast) and No. 7 (existence of nucleus), the systematic position of the alga must be rearranged into Rhodophyceae.

The existence of α , ϵ -diaminopimelic acid which “presents characteristically in most bacteria and the few blue-green algae” was revealed in the present alga by M. B. Allen¹⁾ (1954), and it was also reported by Allen and her co-workers³⁾ (1957) that this substance was not in existence in *Porphyridium cruentum*. And T. Fujiwara and S. Akabori⁶⁾ (1954) reported the existence of the substance in *Chlorella pyrenoidosa*. And so the above-mentioned reports never mean, as the present author believes, that an alga which has α , ϵ -diaminopimelic acid is not a red alga.

If a genus nearest to the present alga is sought among Rhodophyceae, two genera, *Porphyridium* and *Rhodospira*, in the family Porphyridiaceae of Porphyridiales present themselves. The reasons are that on one hand *Porphyridium aeruginosum* is a properly qualified unicellular red alga which contains only phycocyan among Rhodophyceae and on the other no alga can be reproduced by means of endospore-formation but the genus *Rhodospira* among Rhodophyceae.

The morphological comparison among genera in Porphyridiaceae and one more allied family Phragmonemaceae is shown in Fig. 5. As is shown in Fig. 5, the genus *Porphyridium* is not reproduced by means of endospore-formation and its chloroplast is not laminate, but stellate. The genus *Rhodospira* can indeed be reproduced by







Genus	Shape of Cell	Shape of Chromatophore	Phycobilin	fission of cell	endospore	akinetes	sexual reprod.
<i>Rhodococcus</i> (<i>Cyanidium</i>)		spherical	laminate	phycocyan	—	+	—
<i>Porphyridium</i>		spherical	stellate	phycocyan or phycoerythrin	+	—	—
<i>Rhodospira</i>		spherical	discoid	phycoerythrin	+	+	—
<i>Chroothoe</i>		cylindrical	stellate	phycoerythrin	+	—	+
<i>Vanhoeffenia</i>		spherical, ovate or lunate	stellate	phycoerythrin	+	—	+
<i>Phragmonema</i>		cylindrical	irregular laminate	phycoerythrin	+	—	+

Fig. 5. Comparative enumeration of the characteristics of the genera of Porphyridiaceae and Phragmonemaceae.

means of endospore formation, but has many small discoid chloroplasts. It is true that the genus *Phragmonema* has laminate chloroplast, but the shape of the chloroplast is irregular laminate and cells are cylindrical to form a uniseriate filament.

Thus the present alga can not belong to any of the above-mentioned genera, but should be regarded as a new genus among Porphyridiaceae. Moreover the present alga has physiologically peculiar characteristics. Namely this alga is not only thermobiotic but has extraordinarily strong heat- and acid-tolerable nature. This nature can be considered as one of the characteristics to be added to those of the new genus.

As a final conclusion of the above discussions the author wishes to establish a new genus called *Rhodococcus* (non Hansgirg!) and give the present alga a new name, *Rhodococcus caldarius* (Tilden) Hirose gen. nov.

Summary

1. In the present study and the already reported studies of the author, the characteristics of a certain thermobiotic alga which has been named *Cyanidium caldarium*, a member of Cyanophyceae were much more clarified, namely;

(1) Plant cell is unicellular and spherical.

(2) Plant cell contains a single parietal laminate chloroplast which lacks pyrenoid.

(3) Plant is reproduced by means of endospore-formation.

(4) Pigments contained in a cell are chlorophyll *a* and phycocyan and there never exist chlorophyll *b* and phycoerythrin.

(5) Plant never produces starch as its assimilatory product, but may be assumed to be floridean starch, glycogen or soluble dextrine.

(6) Plant cell never shows the differentiation between centroplast and ectoplast as will generally be seen in Cyanophyceae but contains definitely shaped aggregation of chromatins which must be called nucleus.

2. Characteristics of genera of Porphyridiaceae and *Phragmonema* of Phragmonemaceae were compared with one another.

3. By way of conclusion, a new genus, *Rhodococcus*, (non Hansgirg!) was established with *Rhodococcus caldarius* (Tilden) Hirose.

Acknowledgements

My grateful thanks are due to the following five gentlemen: Dr. P. Bourrelly, Director of the Laboratory of Cryptogamy, Natural History Museum, Paris who has given the author many suggestions and kind advice; Dr. L. Geitler, Professor of Wien University, who has given the author invaluable opinions concerning the present alga; Mr. Y. Tsubo who has made any quantity of materials available for me because of his successful culture of the alga and who has assisted the author in

the operations of Abbe's microspectroscope and Beckman's spectrophotometer; Mr. H. Fukasawa who has made many of good preparations of nuclei through Feulgen reaction; and last but not least Mr. K. Takahashi who has endeavoured to determine the assimilatory substances of the present alga.

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摘 要

1. ラン藻類の一員とされ、*Cyanidium caldarium* の名で通っていた温泉藻の1種、イデユコゴメの特徴が、著者のすでに行なった研究と今回の研究により、さらに深く明らかにされた。その特徴を列記すると。

(1) 植物体は単細胞性で、球形を呈する。

(2) 単細胞性の1個体細胞は1個の扁平した布状の葉緑体を有し、葉緑体上にはピレノイドは存しない。

(3) 本植物の生殖はつねに内生孢子形成だけによる。

(4) 細胞内に存する色素の種類は、葉緑素 *a* と、フィコシアニンだけであって、葉緑素 *b* もフィコエリトリンも存しない。

(5) 光合成による炭酸同化産物として、はっしてでんぷんを作ることはない。紅藻でんぷん、あるいはグリコーゲン、あるいは可溶性のデキストリンであるかと想像される。

(6) 原形質の分化として、ラン藻にみられるような、中心質と周辺質との別ではなくて、はっきりした外形をもった、染色質の明らかな集合体、すなわち核とよばれる構造を呈する。

2. チノリモ科のすべての属と *Phragmonema* 科の *Phragmonema* 属の、それぞれの特徴をチャート式に比較した。

3. 最後の結論として、イデユコゴメはラン藻類に所属するものではなく、紅藻に所属すべきものであり、チノリモ科の新属とみなさるべきことを主張し、新属名として *Rhodococcus* なる名前を与え、種名として *Rhodococcus caldarius* (Tilden) Hirose の名を与える。

Miscellaneous Note

Botanical Garden in New China

by Feng-hwai CHEN*

Introduction and acclimatisation of plants in China date back to the Han Dynasty (206 B.C.-220 A. D.). In this respect Michurinism is not new to China. Many crop plants and fodder grasses were introduced and widely utilized at that time. But this work was down solely by individuals and was not as well as organised at the present time what with functioning research centres for botanical science. The botanical garden today is, in a broad sence, not only a research centre but also a recreation ground where natural science education is disseminated among the population.

Soon after the liberation of China in 1949, our government drew attention to the problem of developing botanical gardens in keeping with the needs of the country. The consensus among Chinese botanists and horticulturists is that the development of botanical gardens in China lags behind that of other countries, and that to advance botany more botanical gardens are required. Accordingly, more new gardens have been founded and old ones remodelled. This work was accomplished in the past few years as is amply borne out by the appreciable development of botanical gardens in New China.

The new and newly remodelled botanical gardens are as follows:

Lushan Botanical Garden—the first and the oldest establishment of its kind in

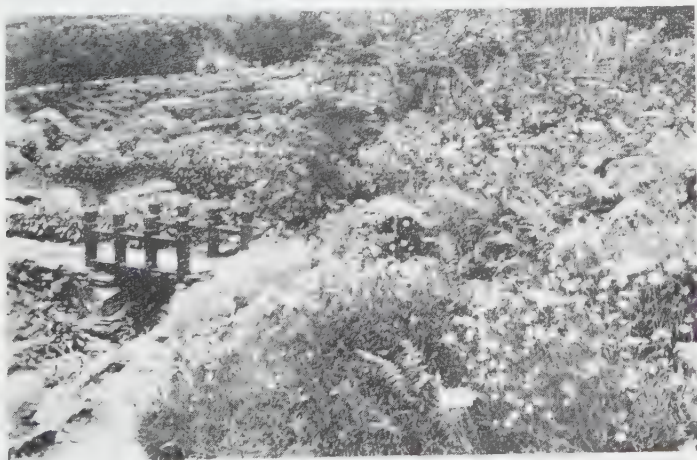


Photo. 1. Abundant and brilliant colours in the rock-garden, the Lushan Botanical Garden

* Nanking Chungshan Memorial Botanical Garden, Academia Sinica



Photo. 2. Japanese Firs, European Spruce and other rare conifers in the woods of the Lushan Botanical Garden

China. Founded in 1934, it belonged to the former Fan Memorial Institute of Biology, Peking. The garden is situated on the top of the well-known summer resort, Lushan Mountain, at an altitude between 11-1200 m. above the sea level, on the upper part of Yangtze River, at the mouth of Pan Yang Lake, near Kiukiang, Kiangsi Province. The garden is bounded by precipices of huge rocks; the gorges afforded a glimpse of the lake—a remarkable beautiful view indeed.

During the period of the Japanese invasion and the reactionary Kuomintang regime many rare and valuable plants introduced from the remote regions of Tibet, Yunnan, Szechuan and other parts of China and abroad were destroyed at random. Good sized specimen trees and beautiful hedge shrubs were cut down for fuel; nume-

rous lily bulbs and rare perennials were lost in this disaster, and well constructed buildings and green-houses were demolished. The garden as a whole was in a tragically desolate condition. In 1950, the second year after the liberation, the garden became a part of the Botanical Institute of the Academia Sinica. Botanists set about reviving their garden. Buildings and roads were repaired; green-houses and propagating shades were reconstructed. The garden has, however, since that time, been laid out anew. Many plants have been rearranged as much as possible according to natural habitat as well as to landscape design.

Being an alpine garden, it contains about two thousand kinds of alpine plants chiefly introduced from West China and European countries. These exotic plants, all cultivated in the open and decorated in various forms, have proved to be well acclimatised to their habit on Lushan.

The arboretum is the most attractive feature of the garden; it consists of more than one hundred species and varieties of conifers and many kinds of broad leaved deciduous trees and shrubs forming a mixed alpine forest. The flowering shrubs like Azaleas, Rhododendrons, Mock-Oranges, Honeysuckles, Spiracas and many other beautiful sorts form a colourful panorama under the woods from early spring to late summer, present a remarkable picture of the garden. The rock garden is the best sight in spring; patches of dainty and exquisite dwarf plants in different shades of

colour set in the crevices of the rocks in an extremely beautiful manner, reveal an aspect of alpine flora. The marshy garden formed below with different kinds of Iris, Daylilies, Buttercups, Sedges, Sweetflags and many others gives an aspect of marshy meadow flora.

Gardeners as well as botanists who come to this garden are attracted by the colour of various plants and the entire garden scenery. It is quite natural that they come to this garden not only for the enjoyment of its scenery but to learn the names of plants and the method of cultivating them.

As regards horticulture, suffice it to say that a proportion of various kinds of exotic plants now found outside of the garden, particularly in the neighbourhood of the province, have been newly introduction into this country from abroad through the agency of this garden. For the Lushan Botanical Garden is reputed chiefly in its successes in plant introduction and landscape designing.

Kunming Botanical Garden—The flora of Yunnan is now admittedly one of the richest in the world. Many garden novelties and industrial plants are still hidden



Photo.3. The arboretum of the Kunming Botanical Garden with trees and shrubs near the pool

in the wilds awaiting cultivation. One of the chief functions of the botanical garden therefore is to introduce and cultivate these valuable plants.

The suggestion to form a botanical garden in Kunming, the capital of Yunnan, was made early in 1940 but it was not effected until after the liberation of China. The garden, situated in the suburbs of Kunming, about 10 miles from the city, is one of the most beautiful spots in Kunming. Chinese botanists had felt all along that a botanical garden should be established here in Yunnan for plant introduction to develop not only botanical science, but horticulture and agriculture as well. Although the garden was not formed until 1950, many useful and interesting plants had already been brought into cultivation.

Yunnan is universally known as the home of the Camellia: the Kunming Botanical

Garden has the best and most varied collection of this flower. Besides, many kinds of industrial plants valuable for spices, drugs, gum-resins and others have recently been discovered and introduced from the wilds. Some of these plants have proved to be useful for essential oils vital for the confectionary and the perfumery industries. It is intended to make this garden not only a botanical centre, but also as a horticulture and agricultural experimental ground.

Peking Botanical Garden—The idea to establish a Peking Botanical Garden was conceived long ago, but was formally inaugurated only in 1954. The local govern-



Photo. 4. The orchid-house in the Peking Botanical Garden

ment allotted about 500 acres of land to Academia Sinica for the site of the garden and it was then organized as a part of the Botanical Institute in Peking. It lies at the foot of the Western Hills near the Sleeping Buddha Temple about 8 miles from Peking.

Owing to the long severe winter and day climate in North China, many useful plants will not grow in Peking or in its vicinity so that it is rather difficult to carry out reforestation. The botanical garden will play an important role in plant introduction and acclimatisation.

Lawn and pasture grasses are badly needed both for grazing and for gardening in North China. There are very few species of either of these grasses in cultivation. Although we have a rich flora of the grass family, many species in this family have not yet been utilized. As a result of experiments, the Peking Botanical Garden has selected a good number of species which have proved to be useful.

Fruit trees like apples, peaches, pears, apricots, grapes, dates and others are famous in North China. We want to improve them in respect to quality and quantity. The garden has started various experiments with this purpose in mind by introducing new varieties from other provinces. Development of ornamental plants such as peonies, crab-apples, lilacs, roses, etc. is stressed. Many collection of these flowers are being cultivated in the garden.

Tropical and subtropical plants in green-houses are arranged according to geographic distribution so that visitors can easily learn the native home of these plants. The Peking Botanical Garden is greatly appreciated by the citizens of the capital of China, particularly the green-house in which many beautiful plants can be seen the year round. The garden will build up a spacious green-house to be used as a conservatory in 1958.

Nanking Chungshan Memorial Botanical Garden—Not far from Chungshan gate and through the main avenue of trees you will find a graceful edifice built in Chinese style in the midst of the woodland garden. This is the newly established Nanking Chungshan Memorial Botanical Garden. Although the garden was only recently



Photo.5. Entrance to the Nanking Chungshan Memorial Botanical Garden. The blooming apricot trees in front of the main building is typical of Nanking scenery.

formed, it was proposed and its name was chosen soon after the death of Dr. Sun Yat-sen, in memory of his revolutionary deeds. A piece of land about 300 acres was reserved but kept idle for about 30 years. After the liberation, the proposal to establish the the Nanking botanical garden was approved by the government and it was soon placed under the direction of Academia Sinica. Work on the garden began in the fall of 1953: the villages and vegetable gardens formerly located on the site of the garden were soon transformed into nurseries and flower gardens. A fine spacious administration building was erected in the summer of 1954.

The arboretum is situated in the central part of the garden and arranged according to geographic distribution. Particular emphasis is given to the flora of the Lower Yangtze. A prominent feature of the flora in this region is its mixed forests consisting of a large proportion of broad-leaved deciduous trees and shrubs of different kinds.

A larger green-house was completed in 1955 containing several hundreds of rare species of tropical and subtropical plants newly introduced from South China.

Professors as well as students come to the garden from different localities from

time to study various branches of botany particularly plant classification. Some come even for special projects on certain groups of plants.

Kwangtung Botanical Garden—This is a newly organized botanical garden on the outskirts of Canton, under the direction of the Botanical Institute, Academia Sinica of South China. The aim of this garden is to introduce tropical and subtropical plants, especially valuable tropical industrial plants, either from China or outside of the country. Bamboo and palm found in the South are rich in species and are worthy of cultivation. Many of these species were brought back from Hainan Island and the borders of Indo-China for cultivation. Ornamental plants of various kinds which bloom all the year round are striking in the South. Many of these plants were recently introduced from different parts of the country or abroad. With the favourable climate of South China, this new garden will soon become an outstanding subtropical and tropical garden in China.

Wuchang Botanical Garden—Wuchang is a lake district of China. Fortunately, the garden was established in this charming beautiful district. The site of the garden is almost completely surrounded by water banked by hills so that it looks very picturesque. This garden was opened in 1956 by Academia Sinica as a research centre of botanical science in Central China. An organized exploration will be conducted jointly by the garden, the Peking Botanical Institute and the Lushan Botanical Garden to West Hupeo and Szechuan to collect and investigate plants.

The above mentioned botanical gardens were recently developed under the direction of Academia Sinica. In addition there is a newly proposed garden to be formed under the guidance of the Hangchow city government in the well-known scenic district of West Lake, Hangchow, Chekiang Province. Prior to the formation of the garden, many varieties of Camellias, Magnolias, Roses, Peonies, etc. are being prepared in nurseries. No doubt, the future garden will play an important role in the park-like Hangchow.

(註). Lushan 廬山, Kunming 昆明, Kwangtung 廣東, Wuchang 武昌

Cytogenetic and Cytoecological Studies on some Japanese Species of *Rubus* IV.

Relation of Polyploidy to Flowering Time, and to Growth Rate

by Taro JINNO*

神野太郎*: 日本産キイチゴ属植物の細胞遺伝, 生態学的研究 IV.
倍數性と開花期ならびに生長率との関係

Received June 28, 1958

Introduction

The studies on the relation between the flowering time and polyploidy, and the growth of plants and polyploidy have already been made by Greis (1940), Eigsti (1942), Löve (1943), Garber (1943), Green (1946) and other workers. According to their reports, the materials used in the investigations are classified into two types, one differing somewhat in the growth and flowering time between a diploid and a polyploid, and the other having no difference.

The present author has observed the flowering time of some Japanese *Rubus*. Moreover, he has studied the growth of leaves and the rooting of some Japanese *Rubus* by stem-cutting propagation. These phenomena are closely connected with polyploidy. This paper deals with the results obtained.

The writer wishes to express his cordial thanks to Prof. N. Shimotomai for his kind direction.

Materials and methods

The materials used in the observations of the flowering time are fifteen species as shown in Fig. 1. Of these species *R. coptophyllus* was collected from Manazuru Peninsula, *R. Fauriei* from prov. Inaba, *R. microphyllus* var. *incisus* from prov. Rikuzen, and the others were all found in prov. Iyo. Of the species obtained in prov. Iyo, *R. ribisoideus* was transplanted from the coastal region, *R. pectinellus* from a place about 800 meters above sea-level, *R. hakonensis* and *R. Wrightii* from a place 500 meters above sea-level, and the others from a place less than 300 meters above sea-

*Biological Institute, Faculty of Education, Ehime University, Matsuyama, Japan 愛媛大学教育学部生物学教室

level. All the materials were grown in the same field for more than a year. The chromosome numbers of these materials had been already counted by the author (1958).

The unfolding time of buds, the growth rate of leaves and rooting of the stem-cuttings were observed. The materials used for the cuttage were ten species as shown in Table 1. Twenty cuttings were taken, about 8 cm. long each with about two buds, per species, and they were set at equal intervals in the wooden-boxes filled with 'Kanumatsuchi' a kind of purmice sand. The wooden-boxes were placed in open air sheltered from the direct sunlight. The investigations were carried out from Feb. 27 to Jun. 15, 1953, and the observation of the rooting was made on the last day after the soil in the boxes was taken out.

Observations

Time of flowering:

The flowering times of ten diploid species and five polyploid species are shown in Fig. 1. The materials used in this investigation have three types of inflorescence: cyme, raceme and solitary inflorescence. In the diploids, the flowering time of cyme is generally later than that of solitary inflorescence. Of the ten diploids six with solitary inflorescences bloomed from the end of March to the end of April, and the remaining four with cymes during the periods between the beginning of April and the beginning of June.

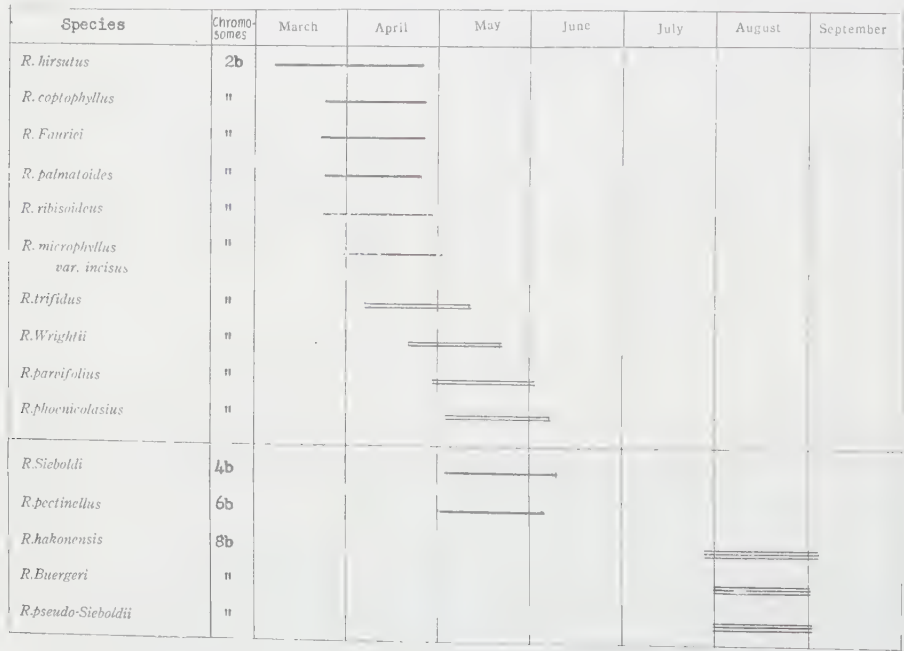


Fig. 1. Flowering times of ten diploids and five polyploids in *Rubus*.
—— Solitary inflorescence
== Cyme
=== Raceme

Table 1. Unfolding and death rates of the cuttings.

Dates		17/III	27/III	6/IV	13/IV	20/IV	27/IV	4/V	13/V	21/V	29/V	13/VII
Chromosome numbers 2n	18 days after	28	38	45	52	59	66	75	83	91	100	100
Species		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
<i>R. palmatoides</i>	14	95.0%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
<i>R. ribisoides</i>	"	80.0	100	100	100	100	100	100	100	100	100	100
<i>R. parvifolius</i>	"	80.0	95.0	100	100	100	100	100	100	100	100	100
<i>R. trifidus</i>	"	50.0	85.0	90.0	95.0	100	100	100	100	100	100	100
<i>R. Wrightii</i>	"	0	75.0	90.0	95.0	100	100	100	100	100	100	100
Diploid average		61.0	91.0	96.0	98.0	100	100	100	100	100	100	100
<i>R. fruticosus</i> var.	28	0	0	42.1	63.2	68.4	78.9	83.3	83.3	88.2	88.2	100
<i>R. Sieboldi</i>	"	0	0	0	0	30.0	40.0	57.9	57.9	85.7	100.0	100
<i>R. pectinellus</i>	42	0	0	0	0	20.0	52.6	84.2	84.2	89.5	100	100
<i>R. sp. (blackberry)</i>	49	5.0	35.0	90.0	90.0	100	100	100	100	100	100	100
<i>R. Buengeri</i>	56	0	0	0	0	30	52.6	88.2	88.2	88.2	88.2	100
Polyploid average		1.0	7.0	26.4	30.6	49.7	64.8	75.1	82.7	90.3	95.3	100

- : death rate

Of the polyploids, *R. Sieboldi* (4b) and *R. pectinellus* (6b), both of which have a solitary inflorescence, bloomed a month or a month and a half later than any of the diploids with the same type of inflorescences.

The flowering time of *R. Buergeri*, *R. pseudo-Sieboldii*, and *R. hakonensis*, all of which are octoploids with racemes, was much later than that of all the other species mentioned above, and it was almost in August. When the flowering time of the octoploids was compared with those of all the others, it was three or four months later than that of the diploids, and two or three months later than that of the tetraploid and the hexaploid.

Unfolding time of buds, and growth of leaves by stem-cutting propagation:

The unfolding rates of buds of stem-cuttings in the diploids and polyploids are shown in Table 1. The percentage was calculated by dividing the number of individuals with unfolded buds by the number of living individuals for each species. As shown in Table 1, the unfolding rate of all the diploids, eighteen days after the settings of the cuttings, was over 50%, except *R. Wrightii*. However, all the polyploids, except the heptaploid blackberry, whose rate was only 5%, did not unfold their buds. Twenty-eight days after the setting, the rates of all the diploids were over 70%, and of them *R. palmatoides* and *R. ribisoideus* were 100%. On the other hand, all the polyploids, except the heptaploid, the rate of which was 30%, did not unfold at that time yet.

In the period from twenty-eight to fifty-two days after the setting of the cuttings, the unfolding rates of all the diploids became 100%. In the same period, however, the unfolding rates of all the polyploids, except the heptaploid, were below 68%, the average being 49.6%, which is the half of the rate of the diploids. The period, in which all the polyploids, except the heptaploid, became 100% in the unfolding rate, was 90 to 106 days after the setting. The period was about twice as long as that of the diploids.

The relation between the growth of the leaves [of the cuttings of the diploids and that of the polyploids is shown in Fig. 2. In the early stage of the cutting, the leaves of the diploids grew more speedily than those of the polyploids, but their growth rate, except for *R. parvifolius*, decreased earlier than that of most polyploids. Though *R. parvifolius* fell in growth rate at a time like the other diploids, it began to grow well in leaves again from about sixty days after the setting. The polyploids had a longer period of growth than the diploids. In the later stage of the cutting propagation, therefore, some polyploids surpassed the diploids in the growth of leaves.

The leaves of the cuttings varied in color from species to species. Seventy days after the setting of the cuttings, the color of leaves were compared with the sample of Color Album (Uemura and Yamasaki, 1946), and their color tones were decided. Of the five diploids three, *R. parvifolius*, *R. palmatoides* and *R. ribisoideus*,

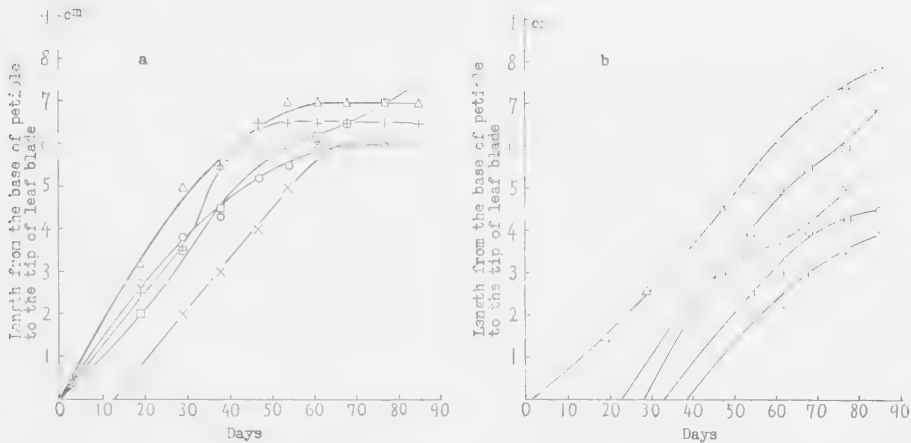


Fig. 2. Comparison of the growth of leaves in polyploids with that in diploids.
a) diploid species: *R. palmatoides*, + *R. ribisoides*, □ *R. parvifolius*, ○ *R. trifidus*, × *R. Wrightii*.
b) polyploid species: × *R. fruticosus* var. (4b), ○ *R. Sieboldi* (4b) + *R. pectinellus* (6b), △ *R. sp.* (blackberry 7b), □ *R. Buergeri* (8b).

were light colored: pale harem green (color number 52) and light diamine green (c.n. 51). And the remaining two, *R. trifidus* aud *R. Wrightii*, were cosack green (c.n. 49). Of the five polyploids one, *R. Buergeri*, was cosack green, but the remaining four, *R. Sieboldi* (4b), *R. fruticosus* var. (4b), *R. pectinellus* (6b) and *R. sp.* (blackberry 7b), had a dark color, bluish cosack green (c.n. 48), which was not seen in the diploids.

The dead cuttings appeared about sixty days after the setting. The death rate became higher as days passed, though it varied from species to species. Dead cuttings of the diploids appeared earlier than those of the polyploids, and the death rate of the diploids was higher than that of the polyploids. In the end, the rate of the

Table 2. Rooting of the stem-cuttings.

Species	Chromosomes	Rates of rooting	Longest roots	Average length of roots
<i>R. palmatoides</i>	2b	0%	0 cm.	0 cm.
<i>R. trifidus</i>	"	10.0	3.2	0.7
<i>R. ribisoides</i>	"	20.0	18.8	4.1
<i>R. Wrightii</i>	"	45.0	15.1	5.3
<i>R. parvifolius</i>	"	85.0	32.7	19.3
<i>R. fruticosus</i> var.	4b	84.2	28.9	20.7
<i>R. Sieboldi</i>	"	30.0	17.7	7.9
<i>R. pectinellus</i>	6b	95.0	21.9	7.8
<i>R. sp.</i> (blackberry)	7b	90.0	24.5	8.6
<i>R. Buergeri</i>	8b	85.0	16.2	9.8

Note: Measured 106 days after the setting of the cuttings.



Fig. 3. Rooting and growth of leaves 106 days after the setting of the cuttings.

a) *R. Buergeri* (8b), b) *R. sp.* (blackberry 7b), c) *R. pectinellus* (6b), d) *R. fruticosus* var. (4b), e) *R. Sieboldi* (4b), f) *R. parvifolius* (2b), g) *R. Wrightii* (2b), h) *R. ribisoides* (2b), i) *R. palmatoides* (2b), j) *R. trifidus* (2b).

former became about twice as high as that of the latter.

Rooting by stem-cutting propagation:

The results of the observations of the rooting of the stem-cuttings are shown in Table 2. As shown in the table, all the materials, except for *R. palmatoides*, produced some roots. Rooting of all the diploids was below 45 %, except that of *R. parvifolius* was 85 %. The polyploids, however, had over 84 % in rooting, with the exception of *R. Sieboldi* (4b), which had 30 %. Generally, the polyploids took root better than the diploids.

All the diploids had roots less than 5.3cm. in length on the average, with the only exception of *R. parvifolius*. On the other hand, the root lengths of all the polyploids were more than 7.8cm.. Generally, the polyploids developed longer roots than the diploids.

Discussion

In the diploid species of *Rubus* used in this investigation, the flowering time of cymes is generally later than that of solitary inflorescences. This phenomenon may be caused by the fact that the peduncle of the cyme takes a longer period to form than that of solitary inflorescence. As far as the author's investigation is concerned, when the flowering times of the diploids and polyploids with the same type of inflorescence are compared, the flowering time of the latter is later than that of the former. *R. Buergeri*, *R. pseudo-Sieboldi* and *R. hakonensis*, all of which are octoploids belonging to subgenus *Malachobatus*, are especially later in flowering than the other species. These octoploid species are distributed from central to west-southern regions of Japan, less than about 700 meters above sea-level. In these regions, no natural hybrid between the octoploid and the other species has been formed. This fact may be due to the physiological isolation, which results from the difference in flowering time.

In the experiment of the stem-cutting propagation, it was confirmed that the polyploids are later in the unfolding time of buds and in the growth of leaves than the diploids in the early stage. This result seems to be similar to the results which Greis(1940), Green(1946) and Löve(1942) observed in the germination of seeds, the

Formation of Starch in Isolated Chloroplasts

II. Experiments with Isolated Chloroplasts Obtained by Plasmolysis*

by Rikizo UEDA**

植田利喜造**：遊離葉緑体のデンプン形成 II. 原形質分離で遊離した葉緑体での実験

Received July 31, 1958

In my previous paper⁹⁾ the viability and function of the chloroplasts isolated by the ultrasonic method were described in detail. In this connection, it is necessary to carry out further studies on this matter using other isolation techniques.

Present report comprises some experimental results on the isolated chloroplast which have been obtained by plasmolytic method.

Materials and Methods

Materials used in this experiments were leaf cells of *Elodea densa*, which were previously made free from starch grains by keeping cells in the dark for a few days.

As already reported by several authors^{3), 4), 12), 13), 14)}, both chloroplasts and protoplasmic fragments could be set free from the mother cytoplasm by plasmolytic method. By treating *Elodea* leaf cells with several kinds of plasmolytica, I could obtain several isolated chloroplasts (solitary or in aggregate) and also protoplasmic fragments with or without chloroplast (Fig. 1.).

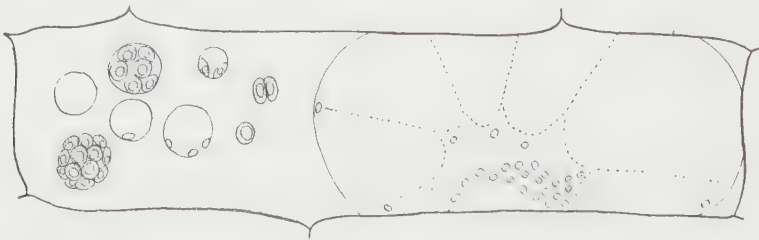


Fig. 1. A schematic representation of a leaf cell of *Elodea densa* plasmolyzed by 0.3 M CaCl_2 (after 6 days), leaving solitary chloroplasts, chloroplast-aggregates and cytoplasmic fragments outside the plasmolyzed protoplasm. Starch grains are contained in solitary chloroplast, in chloroplast-aggregates and in chloroplasts surrounded by thin cytoplasmic layer, but not in the chloroplasts embedded in mother protoplasm or in a large amount of cytoplasmic fragment.

* A part of this study was published preliminarily in Bot. Mag. Tokyo 62 : 62, (1949).

** Botanical Institute, Faculty of Science, Tokyo University of Education, Otsuka, Tokyo, Japan. 東京教育大学理学部植物学教室.

Some of these preparations were stood under diffuse day light inside the window, and other were kept in a dark room, at $27\pm2^\circ$ in both cases. The viability and starch formation of chloroplasts thus isolated were carefully examined.

Results

In order to isolate chloroplasts by means of plasmolysis, and in order to maintain their activity of starch formation, a number of plasmolytica were tested as to their effect upon *Elodea* leaf cells, including KCl, KNO₃, CaCl₂, MgCl₂, Mg SO₄, AlCl₃, KH₂PO₄, and sucrose. All of them were applied at concentrations of 0.5, 0.4, 0.3, 0.2 and 0.1 M, respectively.

Results obtained are summarized in the following table.

Table 1. Effects of plasmolytica on *Elodea* chloroplasts. After plasmolysis, the leaf cells were cultured in a solution of the same plasmolyticum for 6 days at room temperature ($27\pm2^\circ$).

Plasmolytica (in M)	Plasmolysis	Isolated fragment of protoplasm (+ : obtained, - : not obtained)	Isolated chloro- plasts		Chloroplasts in the mother cyto- plasm	
			color	starch	color	starch
Water (control)	-	-	green	-
KCl (0.5)	+	+	green	++	light yellow- ish green	-
(0.4)	+	+	"	++	"	-
(0.3)	-	-	green	-
(0.2)	-	-	"	-
(0.1)	-	-	"	-
KNO ₃ (0.5)	+	+	green	+	light yellow- ish green	-
(0.4)	+	+	"	+	"	-
(0.3)	+	+	"	+	"	-
(0.2)	-	-	yellow- ish green	-
(0.1)	-	-	"	-
CaCl ₂ (0.5)	+	+++	green	+++	yellow- ish green	-
(0.4)	+	++	"	+++	"	-
(0.3)	+	+	"	+++	"	-
(0.2)	+	+	"	+++	"	++
(0.1)	-	-	"	+
MgCl ₂ (0.5)	+	+	green	+	yellow- ish green	-
(0.4)	+	+	"	+	"	-
(0.3)	+	+	"	+	"	-
(0.2)	-	-	"	-
(0.1)	-	-	"	-

MgSO ₄ (0.5)	+	+	light green	—	light green	—
(0.4)	+	+	"	—	"	—
(0.3)	+	+	"	—	"	—
(0.2)	—	—	"	—
(0.1)	—	—	"	—
AlCl ₃ (0.5)	+	+	green	—	light green	—
(0.4)	+	+	"	—	"	—
(0.3)	+	+	"	—	"	—
(0.2)	+	—	"	—
(0.1)	—	—	"	—
KH ₂ PO ₄ (0.5)	+	+	green	++	yellowish green	—
(0.4)	+	+	"	++	"	—
(0.3)	+	+	"	++	"	—
(0.2)	—	—	...	"	"	—
(0.1)	—	—	...	"	"	—
Sucrose (0.5)	+	+	green	++	yellowish green	—
(0.4)	+	+	"	++	"	—
(0.3)	+	—	"	—
(0.2)	—	—	"	—
(0.1)	—	—	"	—

From the data presented above, together with the other observations of the author, treatment with CaCl₂ seems to be most suitable for the present purpose, because the activity as well as the viability of the isolated chloroplasts were preserved for the longest time in cultivation.

The results of my plasmolytic experimentation, which were carried out aiming primarily at the isolation of chloroplasts, are summarized briefly as follows: (1) Among several plasmolytica tested, CaCl₂ seems to be most favorable for the preparation of isolated chloroplasts without damage in an ability of starch formation. (2) Isolated chloroplasts embedded in protoplasm without a nucleus are green and capable of forming starch grains, whereas some chloroplasts accompanied by an appreciable amount of protoplasm do not produce starch grains even in the light. (3) The chloroplasts in the mother protoplasm with a nucleus became yellowish in color, smaller in size, and could scarcely produce starch grain even in the light. Therefore, the existence of the nucleus seems to be responsible for the acceleration of chlorophyll degeneration as well as for the suppressed accumulation of the starch grain in the chloroplasts under the present culture conditions. (4) The chloroplasts in an intact cell, when cultured in tap water, produce some starch only temporarily; but, on further culture the chloroplasts become smaller and smaller, showing that degeneration is taking place.

Discussion

In the previous paper⁹⁾, experiments on starch formation in the chloroplasts isolated by ultrasonic treatment were described. For the same purpose, the author

has tested on a plasmolytic method which is easily applicable under the microscope. This method was once applied by Klebs (1887, 1888) in his classical investigation concerning nuclear functions^{3),4)}. Recently this technique was applied again in several studies on protoplasmic streaming^{13),14)} as well as on nuclear function¹²⁾.

By this method, chloroplasts of the leaf cells of *Elodea densa* could be isolated in solitary or aggregated state, and they were brought into cavities between plasmolyzed protoplasm and cell membrane. With these isolated chloroplasts, starch formation was also clearly demonstrated.

The starch formation in isolated chloroplasts occurs in a satisfactory manner, when they are treated with plasmolyticum containing Ca ion. This fact does strongly support our recognition that Ca ion is essential not only for the maintenance of protoplasmic structure but also for the synthesis of starch in chloroplasts.

Now, the autonomy of the chloroplast in its photosynthetic function is demonstrated by the isolation experiments described above. Besides, the chloroplasts have shown that they can multiply themselves irrespective of cell division^{1),2),5),8)}, and grow sometimes independent of cell growth (unpublished). Moreover, the isolated chloroplasts can move by itself¹⁰⁾, it seems to be able to inherit plasmagones¹¹⁾, and also synthesize starch from glucose-1-phosphate^{6),7)}. All these characteristics seem to suggest that cell-free culture of the chloroplast would be possible in future.

Summary

1. By means of plasmolysis, *Elodea*-chloroplasts were successfully separated from the mother protoplasm. With these isolated chloroplasts, the photosynthetic formation of starch was demonstrated.

2. The photosynthetic activity of the isolated chloroplasts was found to be accelerated of Ca ions added to in the medium.

3. The autonomy of the chloroplast was discussed in relation to the photosynthetic capacity of the isolated chloroplasts.

I should like to express my sincere thanks to Prof. Tomoo Miwa and Prof. Bungo Wada for their invaluable advice and encouragements. Thanks are also due to Prof. Kozo Hayashi for his kind help in preparing the manuscript.

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addendum

After this paper was submitted to the press, the author has received a paper published by S. Hattori and S. Osaki (Zeitschr. f. Bot. 46: 355, 1958), in which from the result of their observations on starch accumulation in the chloroplast they described that it is questionable to draw a distinct line between assimilation starch appearing in chloroplasts and reserve starch in leucoplasts. In this connection it is interesting for us to answer the question how and why the isolated chloroplast accumulates starch grain as a reserve material.

摘 要

前報では超音波作用で生細胞の液胞内に遊離した葉緑体の生存力や機能について報告したが、それに関連して、ここでは他の方法で葉緑体を遊離した場合について研究した。

その大要は次のごとくである。

1. 原形質分離によつてオオカナダモの葉緑体は母原形質から遊離することができた。これらの遊離葉緑体で光合成によるデンプン形成が証明された。
2. 遊離葉緑体の光合成はカルシウムイオンの存在で促進された。
3. 遊離葉緑体の光合成能に関連して葉緑体の自律性について考察した。

Analysis of Gas Contained in the Fruit of Indian Lotus Plant

by Kiyonobu TOYODA*

蓮果中の気体の成分分析

Received August 22, 1958

Twenty-five old fruits of Indian lotus plant (*Nelumbo nucifera* Gaertn.) were collected from the mud of the Genpei pond of the Hachiman Shrine at Kamakura City by the writer in early July, 1957. This pond is said to be made about seven hundred years ago and Indian lotus plants being planted at the same time, according to a historical document. These fruits, therefore, are considered as old as one year at least to seven hundred years at most. The place where these fruits were found is not, however, the lowest layer of the mud, and most of these fruits have preserved epidermis and remnants of styles, although old fruits have no epidermis in general, but a luster and hardly styles. These fruits, therefore, seemed not to be so old and it is likely that they are a mixture of various fruits of ages above one year.

There is a small cavity of about 0.15 ml. in the interior of the fruit, and a green plumule exists in this cavity. The pericarp is very hard and is not generally permeable to water and gas. As it was at first considered to be very important to analyze gas contained in the fruit, the writer made some experiments. The writer worked out a particular method for taking out and analyzing the gas contained in the fruit. Comparing the data with those in new and old fruits in low and high temperatures, the writer studied the metabolic activity of the fruits of Indian lotus plant.

Material and Methods

The fruits used for gas analysis were the old ones collected in the Genpei pond at Kamakura, the rest were the new ones which were obtained from receptacles found in the same pond, and in lotus fields at Fukazawa in Kamakura and at Ohba in Fujisawa.

The hard palisade layer of pericarp was filed away first, then an orifice was drilled and which was filled with liquid paraffin. The fruit was immersed in liquid paraffin filled in a vessel and the needle of an injector was pierced into the fruit. Then drawing the piston, the gas contained in the fruit was sucked into the injector by replacing liquid paraffin (see Fig. 1). The gas in the fruit was thus collected by suction one by one, taking each time a quantity of one to two ml. and the injector

* Fujisawa Higher School attached to Nihon University, Fujisawa, Kanagawa Pref., Japan
日本大学藤沢高等学校

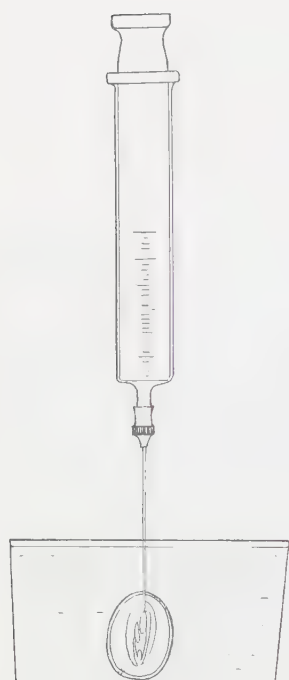


Fig. 1 Gas contained in the fruit is obtained in an injector by replacing liquid paraffin.

was immersed in water, the needle being detached then from the injector and put a rubber cap on the tip. Another injector with a rubber tube (1-1.5 cm. long) to the tip was also immersed in the water and the gas in the first injector was then removed into the second and used for gas analysis.

As regards gas analysis, there are various methods, but the writer devised a simple method suitable for micro-analysis based on absorption as follows: an injector holding the gas was used, besides a micropipette, a Z-shaped micropipette which was specially made, a short glass tube swollen in the middle part (see Fig. 2, 3), a connecting rubber tube, a fixing apparatus and a thermostat.

Carbon dioxide: Content of the gas is measured by absorbing it with potassium hydroxide. The injectors holding the gas and potassium hydroxide solution were put in a thermostat beforehand. The glass tube swollen in the middle part was connected with the micropipette by a rubber tube and the tip of the micropipette was steeped in potassium hydroxide solution and the solution was sucked up beyond the glass tube swollen in the middle part by another injector.

The whole was placed on a plate and the tip of the micropipette was raised a little, and the rubber tube connecting the glass tube and the injector was pinched with a pinch-cock. It is necessary to settle the whole with a fixing apparatus in that position. The injector was replaced rapidly with the injector holding the gas above mentioned, the piston was pushed a little so that the reagent might remain at the tip of the micropipette and fixed at the position. The injector was shaken from time to time ordinarily for 5-10 min.. The decrease of the gas volume was measured.

Oxygen: The decrease of the gas was measured by absorbing oxygen with an alkaline pyrogallol solution. The potassium hydroxide solution in the injector was excluded just after measuring carbon dioxide and rubber cap was put on. A Z-shaped



Fig. 2 Carbon dioxide is measured by absorbing it with potassium hydroxide using an injector, a glass tube swollen in the middle part and a micropipette. The arrow shows the place pinched by pinch cock.

micropipette was connected with glass tube swollen in the middle part and an alkaline pyrogallol solution was sucked in. The quantity of oxygen absorbed was measured by the method described above.

Correction for errors: Errors caused by the change of temperature, atmospheric pressure and vapor pressure is to be calculated according to the formula respectively, it also depends upon the volume of the gas, solution and vessel, and therefore becomes very complicated making it difficult to calculate correctly. To avoid these difficulties the writer used a micropipette and an injector holding the air of the same volume as the gas and a red solution of the same volume as the reagent solution as control. A reading was made at the beginning and the writer worked with this reading as a standard during a single experiment.

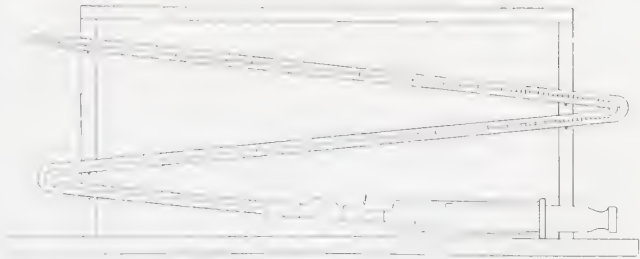


Fig. 3 Oxygen is absorbed by an alkaline pyrogallol solution using a Z-shaped micropipette, an injector and a glass tube swollen in the middle part. The arrow shows the place pinched with a pinch-cock.

Results

The results of the analysis of the gas obtained from the old and new fruits are as follows¹⁾:

Table 1

Old fruits (experimented in early July): Seven or eight fruits were used. The quantity of gas was about one ml. in each experiment.

	CO ₂ (%)	O ₂ (%)	CO ₂ + O ₂ (%)
	0.7	19.8	20.5
	0.9	19.1	20.0
	0.6	19.7	20.3
Average	0.7	19.5	20.3

Table 2

New fruits (experimented in early and late Sept.)

	CO ₂ (%)	O ₂ (%)	CO ₂ + O ₂ (%)
	1.7	17.9	19.6
	1.2	18.6	19.8
	1.5	18.7	20.2
Average	1.5	18.4	19.9

In order to research the change affected by the time, the fruits allowed to stand at room temperature were analyzed every month. Eight or nine fruits, and the gas of about one ml. were used in every experiment; the experiments were repeated from two to eight times (see Fig. 4).

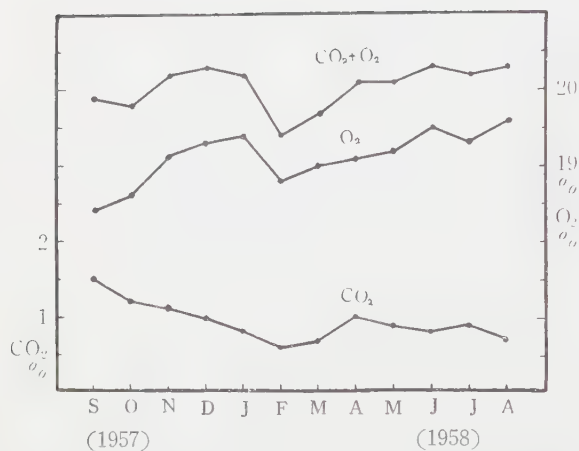


Fig. 4 Monthly change of the values of the gas analysis in newer fruits (from September to August).

The content of CO_2 reads on the left side of ordinate, the content of O_2 on the right.

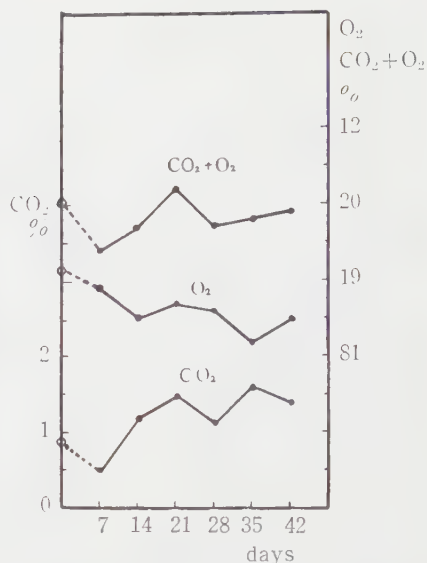


Fig. 5 Average content of O_2 , CO_2 and $\text{CO}_2 + \text{O}_2$ in the fruits for various days at 0° . The marks on the left ordinate indicate average content of O_2 , CO_2 and $\text{CO}_2 + \text{O}_2$, respectively, in the newer fruits from November to May.

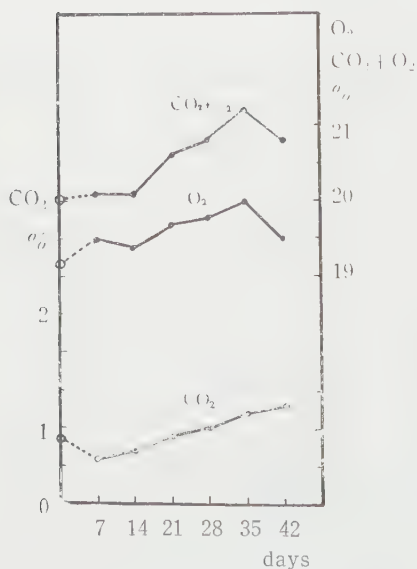


Fig. 6 Average content of O_2 , CO_2 and $\text{CO}_2 + \text{O}_2$ in the fruits for various days at 30° .

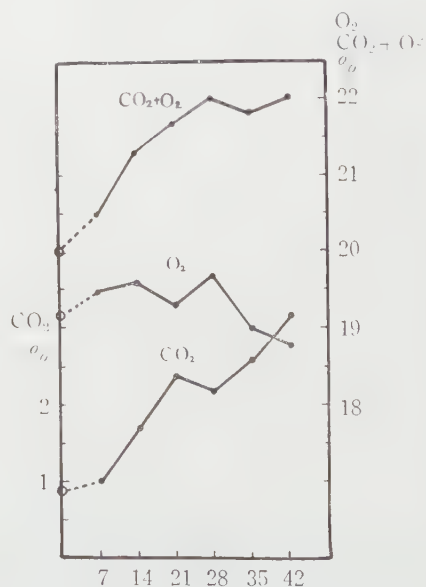


Fig. 7 Average content of O_2 , CO_2 and $\text{CO}_2 + \text{O}_2$ in the fruits for various days at 60° .

To see the effects of temperature, the fruits were preserved for various days at 0°, 30°, 60°, and these experiments were carried out from November, 1957 to May, 1958. The results are shown in Figs. 5, 6, and 7.

The fruits kept in the thermostat at 60° for 30 days were taken out and put into a beaker filled with liquid paraffin at room temperature in February and March. During the experiments no bubble was observed to come out from the pericarp, so it may be impermeable to gas. Taking out and analyzing the gas in the fruits, the results are as follows:

Table 3

Days	CO ₂ (%)	O ₂ (%)	CO ₂ +O ₂ (%)
7	0.6	20.4	21.0
14	0.5	20.7	21.2
21	0.7	20.5	21.2

Same experiments were carried out in the period from March to June, the results being as follows:

Table 4

Temperature	Days	Days (soaked in liquid paraffin)	CO ₂ (%)	O ₂ (%)	CO ₂ +O ₂ (%)
60°	30	7	0.6	19.6	20.0
60°	30	30	0.9	20.1	21.0
60°	45	7	1.1	20.1	21.2

These experiments at 60° were carried out further up to 42 times, however the results obtained seemed not to differ from those described above. So the writer refrained from recording them here. Experiments were made with the ones soaked in liquid paraffin after being placed at 60° 22 times repeatedly. The whole average content of carbon dioxide and oxygen are as follows:

Table 5

	CO ₂ (%)	O ₂ (%)	CO ₂ +O ₂ (%)
The ones placed at 60°	2.2	19.4	21.6
The ones soaked in liquid paraffin after being placed at 60°	0.8	20.4	21.2

Discussion

As is to be seen from the results of these analyses, there are some differences between the old fruits collected in the mud and the newer ones taken from the receptacles. Carbon dioxide is present in the newer fruits a little more than in the old ones, while oxygen is less. It may be regarded to come from the fact that in the newer fruits, moisture is more and respiration is more active, and they are subject to the ampler range of temperature change than the older ones embedded

in the mud about 1m. deep.

In the newer fruits, however, some changes occurred, in the component of gas after a lapse of time at various temperatures.

Since October the quantity of carbon dioxide was, as a rule, decreased while oxygen increased generally, and after several months the newer fruits became to show almost the same gas component as old ones. In February, content of oxygen decreased fairly as shown in Fig. 4.

Researching the change of gas components in the newer fruits at various temperatures, 0° , 30° and 60° , carbon dioxide had an increasing tendency after a temporary decrease at the initiation, and oxygen decreased at 0° as shown in Fig. 5. Carbon dioxide had also an increasing tendency after a decrease, and oxygen increased a little and did not change so much at 30° (see Fig. 6). Carbon dioxide increased fairly and oxygen decreased after an increase at 60° as shown in Fig. 7. If the fruit is kept in this state, carbon dioxide shall extremely increase and the fruit should be dead at last.

In perfectly ripened fruits of Indian lotus plant, the pericarp did not generally allow water to penetrate. To confirm this fact, the fruits were kept in the liquid paraffin at room temperature after having been kept in a thermostat at 60° for 30 days, no bubble seemed to occur. Nevertheless, carbon dioxide decreased a good deal, oxygen increased as shown in Tables 3, 4 and 5; it was a very remarkable thing.

Whether the pericarp is permeable to water and other substances or not, we have some reason to deny. It is evident, if it allows water to permeate, the fruit should either swell followed by decaying or germinate as the writer experimented previously²⁾.

So the writer is of the opinion that metabolism is being carried out in the inner part of the fruit, though very much slowly. The embryo respire extremely slowly, though carbon dioxide is present to lesser and oxygen to more extent in the old fruits than in the newer ones. Furthermore, carbon dioxide decreased and oxygen increased in the fruits that had been kept in liquid paraffin after being kept at 60° at various time lengths.

The life span of Indian lotus fruits has been generally believed to be fairly long, but no one has ever been possible to explain the reason. In this connection the fact disclosed in this article may bring some light on the problem, because it has shown remarkably unusual ratio of carbon dioxide and oxygen content in the fruit.

The writer wishes to express his sincere gratitude to Professor Shizuo Hattori of the University of Tokyo for his kind guidance and revising the manuscript.

Summary

1. The gas contained in the old fruits of Indian lotus plant which were collected from the mud of a pond was taken from them by replacing the gas with liquid paraffin by an injector,

2. The gas was analyzed by a method of writer's own.

3. The gas contained in the newer fruits was also analyzed, and there were found more CO_2 and less O_2 than in the old ones. Some change was, however, observed in the content of CO_2 and O_2 after a lapse of time. After several months, newer fruits became to have almost the same gas ratio with the old ones.

4. Investigating the change of gas components in the fruits at various temperatures, 0° , 30° and 60° , CO_2 had an increasing tendency after a decrease and O_2 decreased in general at 0° . CO_2 had also an increasing tendency after a decrease and O_2 increased a little at 30° . At 60° , CO_2 increased fairly and O_2 decreased.

5. The fruits which had been kept in liquid paraffin after being at 60° for some time, did not evolve gas through the pericarp. The result of gas analysis which was made of these fruits, CO_2 showed a decrease and O_2 increased.

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摘 要

1. 蓮の古果のガス組成を、その果実の中心部に含まれてゐるガスが、流動パラフィンに置換されてから分析した。

2. 蓮の古果のガス組成を、その果実の中心部に含まれてゐるガスが、流動パラフィンに置換されてから分析した。

3. 新しい果実に含まれてゐるガスも分析した。この結果、新しい果実では古い果実に比して炭酸ガスが多くて酸素が少ないことがわかつた。

しかし、新しい果実に含まれてゐるガスを毎月分析してみると、時の経過によって変化が見られ、炭酸ガスは減少し、酸素は増加し、 60° では炭酸ガスは減少し、酸素は増加する傾向がある。

4. 0° , 30° と 60° の各温度における果実内のガス組織の変化をみると、 0° では炭酸ガスは減少したあと増加する傾向があり、酸素は一般に減少した。 30° では炭酸ガスは減少したあと増加し、酸素は少し増加した。 60° では炭酸ガスはかなり増加し、酸素は減少した。

5. 60° にある期間においてあと流動パラフィンにつけた果実をみると、果皮よりガスの放出はみられない。これらの果実内のガスを分析した結果、炭酸ガスは減少し、酸素は増加するというきわめて注目すべき結果が現われた。

The Effect of Light on Gamete Liberation in *Monostroma*

by Ikuko SHIHIRA*

志平依久子・ヒトエグサの配偶子放出と光の影響

Received August 23, 1958

Many species of Ulvales periodically liberate spores, sexual or asexual. The periodicity of the liberation of spores is correlated with the periodicity of spore formation. The capacity for liberation appears after the cells have completely matured, but fertile cells, even though they may be fully ripe, do not always liberate the spores which they contain. The conditions that induce liberation seem to be different from those that promote maturation.

As to the other sea-weeds which grow in the upper part of the intertidal zone, the factors controlling liberation have been considered to be the change of fronds from desiccation to immersion for *Enteromorpha* and *Gloipeltis*,⁹⁾ and from dark to light for *Monostroma* and *Cutleria*. Light has been considered to have a significant influence on gamete liberation because this phenomenon in nature usually occurs at dawn. Light was found to have the greatest effect, but it was not indispensable to activate the gametes.

Monostroma was used to study the influence of light and dark on gamete activation. The substance responsible for gamete activation is suggested to have a remarkable photosensitivity, especially for the blue light, contrary to the seed plants, in which the longer wave length was more effective upon the germination of seeds and the initiation of flowering^{1), 2), 8)}.

Material and Method

Monostroma nitidum growing luxuriantly on the rocks along the beach in the vicinity of the Misaki Marine Biological Station was used for the experiment. Every year the algae appear in November in the field, and produce gametes from January until they disappear in the end of May. Their maturation shows a distinct periodicity in March and April. The experiments were performed in most cases during April and May. Gamete formation in the cells is easily recognized with the naked eye, by the yellow color. Such mature frond was collected, and the fertile part of frond was cut into small pieces of about 3–5mm². Fragments from the same frond were mixed, and were kept separately in petri dishes filled with sea-water. As the source of artificial light, 100-watt incandescent lamp or two 20-watt fluorescent lamps were used.

* Marine Botanical Institute, Fisheries Department, Faculty of Agriculture, the University of Tokyo, Tokyo, Japan. 東京大学農学部水産植物学教室

The dishes were completely covered with tin boxes, so that all light was excluded during the period of darkness. Agar-filters prepared by Dr. S. Sakurai (Scientific Research Institute) were used to provide light of various wave lengths.

Experiments and Results

In the preliminary observations, it was found that a frond with ripe gametes liberated them usually at dawn under laboratory conditions. However, fronds kept under illumination throughout the night did not liberate gametes, and this phenomenon was often verified by field observations. These findings suggested that the change from dark to light is most important, or necessary to induce liberation of gametes. Some experiments concerning these problems were carried on as described below.

Exp. 1. Effect of dark upon gamete liberation: When fertile fronds were exposed to light, after a sufficient exposure to darkness, they liberated gametes in 5 minutes. All of these swam to the light side of the vessel. These gametes were removed with a glass pipette, and the frond was left in the light. No more gametes were liberated even after several hours. Thus, after a sufficient period of darkness, light appears to act solely as a trigger agent.

Exp. 2. Correlation between the length of the time gametes are contained in cells and the length of the dark period necessary to cause liberation: Fronds kept in the light for various periods, 1 hr., 7 hrs., 13 hrs. and 19 hrs., were removed to the dark for various length of time. A long dark period required for activation, when the time in the light was short, becomes shorter as the light period was lengthened, and finally becomes unnecessary (i. e., as the state of "maturity" of the gametes advanced).

Table 1. Correlation between length of time when gametes are contained in cells (pretreatment) and length of dark period (treatment).

+ : quantity of gametes liberated

Pretreatment in hrs.					
Treatment in min.		1	7	13	19
1		—	—	—	+
5		—	—	—	++
10		—	—	+	++
30		—	+	+++	+++
60		++	++	+++	+++

Exp. 3. Non-additive nature of dark periods: Thirty minutes of darkness were inserted into a light period of 6 hrs. in two different ways: (A) the whole 30 minutes dark period was applied at the end of the light period, and (B) 5 min. dark periods

were inserted 6 times at 1 hour intervals (Fig. 1.). And also (C) 10 min. dark periods were inserted 6 times in the same way as (B). Gamete liberation took place in (A), while it failed to occur in (B) and (C), indicating that subminimal dark periods cannot be added to produce an adequate stimulus.

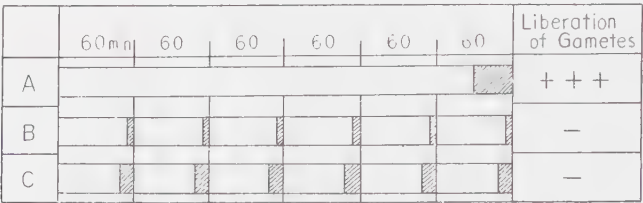


Fig. 1. Ineffectiveness of darkness inserted into a light period.
Blank : light period
Hatched : dark period

Exp. 4. Effect of darkness interrupted by light: The frond for which a 15 min. dark period was not sufficient to cause liberation, but a 30 min. dark was sufficient, was used for this experiment. Many pieces of fertile fronds in petri dishes were put into the dark at the same time. Various lengths of light period were inserted into the middle of a 30 min. dark period. Thirty minutes of continuous darkness induced liberation of great numbers of gametes. Inserted light periods of short length, 1 min., 5 min. and 10 min., resulted in a slight decrease of gamete liberation. When 20 min. or longer light period was inserted, the amount of liberation decreased considerably. Thus it appears that when the necessary dark period is interrupted by the light, the effect of the former dark period is cancelled.

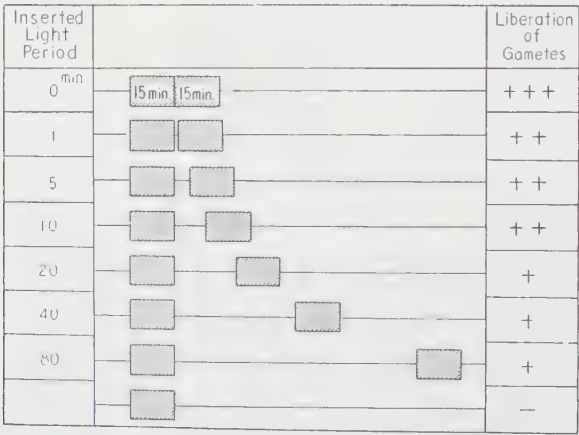


Fig. 2. Effect of darkness interrupted by light.

Exp. 5. Light periods following exposure to dark: (1) Samples of material illuminated continuously were exposed to dark periods of 15 min., 30 min., 1 hr. and 2 hrs., respectively. After these dark periods, 1 sec., 10 sec. or 30 sec. irradiation (2,500 lux) was applied and the material was returned to the dark. After about 30 min., all the pieces of the fronds were removed from the petri dishes in the dark, and the amounts of liberated gametes compared. A series of experiments were started in all lots at the same time, but in another series of experiments the irradiation was applied at a definite time. Both series of experiments gave the same results. After a sufficient length of darkness, liberation is induced by 1 sec. irradiation. Shorter dark periods required longer light periods.

Table 2. Correlation between the quantity of gamete liberated and length of dark periods and of light periods following exposure to dark.

Dark in min.	Light in sec.			
		1	10	30
15		—	—	—
30		—	+	++
60		+++	+++	+++
120		+++	+++	+++

(2) Fronds kept in light for various periods such as 3 hrs., 6 hrs. or 9 hrs. were exposed to dark periods of 1 hr., followed by irradiation of 1 sec. or 1 min. The quantity of liberated gametes increased in proportion to the length of the period of illumination. One hour of dark followed by 1 sec. light becomes sufficient to induce liberation as time elapses after the appearance of gametes in the cells.

Table 3. Effect of dark periods of 1 hour, followed by irradiation of 1 second or 1 minute (treatment) in fronds kept in light for various periods (pretreatment).

Treatment	Pretreatment in hrs.			
		3	6	9
1 sec.		—	++	+++
1 min.		+	++	+++

(3) Light intensity was altered by changing the distance from the light source to the irradiated petri dish. Under such different light intensities as 4000, 400 and 40 lux, fertile fronds which had been kept in darkness were irradiated for 1 sec., 10

sec. or 30 sec. Under 40 lux, liberation did not occur until after even longer exposure, while under 4,000 lux, abundant gametes were liberated after even 1 sec. irradiation. The quantity of gametes liberated after sufficient duration of darkness was proportional to light intensity and length of light period within certain limits.

Table 4. Correlation between the quantity of gametes liberated and the intensity of exposure to light period following to darkness.

Light duration in sec.	Light intensity in lux	4000	400	40
1		+++	+	-
10		+++	++	-
30		+++	+++	-

Exp. 6. Effective wave length: (1) After sufficient dark exposure, the materials were exposed for 30 sec. to light of various wave length of equal energy. As light of high intensity could not be obtained through the filters, gametes in cells did not become sensitive to short exposures. Gametes were liberated under the light passing through a blue filter (430-450 $m\mu$), while they failed to be liberated under green light (500-580 $m\mu$). It appears that only light having a wave length shorter than 500 $m\mu$ is effective in causing gamete liberation. Liberation can take place under the light of an ultra-violet sterilizer lamp. Since its light includes some light of 430-500 $m\mu$ wave length range, it could not be concluded that ultra-violet was effective, but at least it is certain that it is not inhibitory. Light of wave length shorter than 500 $m\mu$ is necessary to induce liberation of the gametes.

(2) Instead of dark treatment, materials were illuminated with lights of various wave lengths. One or several hours later, they were exposed to natural light. Only the material kept under the filter of 430-540 $m\mu$ wave length failed to liberate gametes

Table 5. Effective wave length in causing gamete activation

Wave length of filters ($m\mu$)	Liberation of gametes
430-540	+
500-580	-
540-640	-
580-680	-
510-750	-
580-750	-

in natural light, like the all light controls. The light period under the other filters causing the liberation of great quantities of gametes on subsequent irradiation had an effect on liberation similar to that of darkness.

(3) Only light of wave lengths shorter than 500 m μ was effective as interposed light, in nullifying the effect of a former dark period, as described in Exp. 4.

Discussion

Many studies have reported that initiation of flowering in seed plants and germination of seeds are correlated with dark and light. The fact that swarmers of some sea-weeds are generally liberated in the morning has been observed by many investigators and it has been considered that light, following the period of darkness, acts as the stimulus for liberation.

According to Ingold and Dring,³⁾ alternating exposure to darkness and light is necessary for spore discharge in fungus, *Sordaria*, and the blue light is the most effective. The author's observations on the Ulvaceous algae agree with those above. They show that the swarmers in this group also show the strongest phototaxis in the blue light, that is, they move most rapidly under the light of wave lengths below 500 m μ . Therefore it seems possible that the same kind of photoreceptor mechanisms occur in both the algae and fungi. This possibility is being investigated.

On the basis of the results reported in the study, following scheme is suggested; Activation of gametes depends on two reactions A and B. Reaction A occurs in the absence of blue light; once it is completed, reaction B can occur after even momentary exposure to blue light. Light of other wave length is ineffective. Since Reaction A (effect of darkness) was found to be inhibited by low temperature (0 - 2°) for several hours (although it eventually appeared after several days), it is obviously some metabolic reaction which can proceed in the dark, while reaction B must be a photo-sensitive one. This situation is almost similar to the case reported on the germination of *Nigella* seed⁶⁾. It is further suggested that admission of light before reaction A is completed, causes it to be reversed. Many phenomena consisted of two reactions stated above, have been observed in seed plants especially in the floral initiation, or the germination of seeds, or spores or some land plants^{4), 5), 6), 7)}. Such momentary light reaction as B closely resembles to the stimulant light promoting the germination of Tobacco seed, "Bright Yellow".

The finding that increasingly shorter exposures to darkness become effective as the state of "maturity" advances (Table 1) suggests that reaction A can proceed slowly even in the light. In this respect the gametes of *Monostroma* are similar to the swarmers of *Ulva* and *Enteromorpha* which are activated by other changes, such as immersion from desiccation.

Summary

It has been determined that exposure to light following a period of darkness ac-

tivates the immobile gametes in the cells of *Monostroma*. This report deals with a correlation between such activation and the light conditions concerned.

1. After a sufficient period of darkness, exposure to light appears to act as a trigger agent.

2. The length of darkness necessary to cause liberation becomes shorter as the "maturity" of the gametes advances, and finally activation can occur without exposure to darkness.

3. The necessary duration of darkness is not additive.

4. When the necessary dark period is interrupted by exposure to light, its effect is cancelled.

5. Light periods following exposure to darkness: After a sufficient exposure to darkness, liberation was induced by 1 sec. irradiation, while insufficient dark periods required longer exposures to light. The condition of 1 hr. exposure to darkness followed by 1 sec. light becomes sufficient to induce activation as the gametes become more "mature". The quantity of gametes liberated after sufficient exposure to dark is proportional to the light intensity and length of light period within certain limits.

6. Light of wave length shorter than 500 $m\mu$ is necessary for liberation. Light of wave length that was ineffective for liberation had an effect similar to that of darkness. Light of short wave length was effective in nullifying the effect of a preceding dark period.

From the above facts, it is suggested that in *Monostroma* the reaction which leads to activation of the gametes consists of two parts. The first reaction proceeds at a suitable temperature in the absence of blue light, and when this reaction is complete, the second reaction is induced by momentary exposure to blue light.

The author is indebted to Ass. Prof., S. Arasaki through her algological works. The grateful acknowledgements are made to Prof. T. Miwa and to Dr. S. Ishikawa, Tokyo University of Education, for their valuable and helpful advice and encouragement, and to Dr. J. Dan for suggestions concerning the preparation of the manuscript. Also her deep gratitude is due to the staff of the Misaki Marine Biological Station for their kindly and warm support.

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摘 要

緑藻ヒトエグサの配偶子の活性化は、暗期を必要とする。暗期の長さを短くして全体が放出にじゅぶんな長さになるように暗期を与えてもその効果はあらわれなかった。すなわち断続的な暗期は配偶子の活性化に効果的でない。暗期の長さ、暗期の長さの平方、暗期の長さの立方と照度に関係があるが、暗期がじゅぶん長いときには 400 lux, 1 秒でまったく効果をあらわし、いちじるしい光感性をしめした。なおこの光効果は、500 mμ 以下の短波長光でもたらされ、それ以上の波長の光は効果がない。

配偶子の活性化は、暗反応—青色光以外の光下でおこなわれる metabolic な反応—とこれが完成したときから起る放出反応—とからなる。この二つの反応は、それぞれ異なる光の条件下で起る。配偶子の活性化は、高等植物の光周期反応とよく類似するものとおもわれる。有効波長については高等植物ではがいしてこの逆の場合が多い。Ingold & Dring (1957) によれば、子実菌 *Sordaria* の胞子放出には、闇と光が必要で、青色光がもっとも有効である。上記のように、ヒトエグサの配偶子の活性化における光効果はこの *Sordaria* の場合と共通性がみられる。

Effect of Chlorophyllins on Proteolytic Enzyme

by Yoshinobu OSAWA*

大沢義信*; 蛋白分解酵素に対するクロロフィリンの影響

Received August 29, 1958

In the course of study on chlorophyll in wound healing and in suppurative diseases, Bowers¹⁾ suggested in 1947 the bacteriostatic action of chlorophyllin. This has been ascertained and extended further by many workers^{2~8)}. Kimura and Nakamura (1954)⁹⁾ found an inhibitory effect of chlorophyll derivatives upon the growth of *Candida* cells. The differential inhibition of viral hemagglutination by chlorophyllin was also reported by Dunham (1954)¹⁰⁾.

Concerning the effects of these compounds on catalytic activity of enzymes, only a few reports have appeared heretofore. Rapp (1949)¹¹⁾ found that the water-soluble chlorophyll blocked certain enzymatic activities of human saliva. Recently, an anti-hyaluronidase action of chlorophyll was described by Vecchio (1955)¹²⁾. Wasielewski and Albrecht⁶⁾ observed, in their studies on *Bacterium proteus*, that the proteolytic activity of this organism was considerably blocked by added chlorophyllin. On the other hand, an accelerating effect of chlorophyllin on digestion of serum and coagulated egg-white by pancreatic preparation was described by Zirm and his collaborators (1953)¹³⁾ in the course of their studies on the formation of chlorophyllin-protein complexes. Since some inhibitors for tryptic enzyme have been demonstrated in pancreas¹⁴⁾, serum¹⁵⁾ and egg-white¹⁶⁾, it is highly desirable for us to study the effect of chlorophyllin on proteolysis using purified enzymes and substrates. The present paper comprises some experimentations carried out with crystalline samples of both trypsin and some chlorophyllin compounds. Crystalline trypsin¹⁷⁾ is rather stable on dialysis and is known to have neither characteristic prosthetic group nor coenzyme. Besides, various accelerators^{18), 19)} and inhibitors^{14~16), 20~29)} of this enzyme have been already reported. For these reasons, the crystalline trypsin has been used as test enzyme, and the studies on action mechanism of chlorophyllins toward proteolytic activity of trypsin have been carried out by the present writer since 1955.

Meanwhile, Morishita *et al.* (1957)³⁰⁾ studied the stimulating effect of various metallo-chlorophyllins upon hematopoietic capacity and stated that both metals and porphyrin structure were essential to the hematological effect. This is due to the formation of certain complex compound between serum protein and chlorophyllin, which was shown recently by Zirm *et al.*¹³⁾ who used labelled chlorophyllins. In 1954,

*The Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Kiyose, Tokyo, Japan. 日本結核予防会結核研究所

Kajita and Uchimura³¹⁾ demonstrated that hematin combined easily with histidine in their dissociated state. In view of these facts, it is tempting for the present writer to know whether the chlorophyllins exert their effect upon trypsin as a metallo-porphyrin itself or in some other forms. He³²⁾ already suggested that the inert complex formation constitutes a plausible mechanism responsible for the inhibitory action of chlorophyllin against trypsin. It has not been ascertained that the iron participates in the linkage between iron-chlorophyllin and trypsin, as already known in hemoglobin, cytochrome, catalase and peroxidase. And, an important radical in protein moiety of hemoglobin essential for the metalchelation consists probably in imidazole ring of histidine^{33~38)}. Besides, this and/or some other groups (SH, NH₂, etc.) are also assumed in cytochrome c³⁹⁾ and hemoglobin^{40~43)}. That the carboxyl groups of protein are also responsible for heme-affinity was reported by Philipp⁴⁴⁾ and Haurowitz⁴⁵⁾ on hemoglobin, by Theorell and Paul⁴⁶⁾ on horse-radish peroxidase, and by Lemberg and Legge⁴⁷⁾ on hematin-albumin complex. In view of the structural resemblance between chlorophyllin and heme (or hematin), it is interesting for us to study the mode of association between chlorophyllin and trypsin, especially because of the fact that an enzyme constitutes the partner of combination.

Green⁴⁸⁾ found that some protein substances inhibited trypsin in the manner which differed from the usual type of competitive inhibition. Besides, he studied in detail on the nature of interactions found in Ca ion, di-isopropyl fluorophosphate, mercuric chloride, protein-like inhibitors, and substrate. It seems to the present writer that the mode of action of chlorophyllin on trypsin should be clarified by stoichiometric and spectrochemical investigations about the interaction between chlorophyllin and other substances in the manifestation of tryptic activity.

According to Gorini *et al.*⁴⁹⁾, trypsin is protected from its autolysis by divalent cations such as Ca⁺⁺ and Mn⁺⁺. The effect of these ions is, after them, to be ascribed to a decrease in lability of the substrate molecule.

On the other hand, the proteolytic enzymes are shown to be denatured by various treatments, e.g., heat-shock^{50), 51)}, alkali-treatment⁵¹⁾, β or γ irradiation and ultraviolet^{52), 53)}, whereas Ram and his associates⁵⁴⁾ and Herriott *et al.*⁵⁵⁾ observed the stabilization of certain proteolytic enzyme after its acetylation. This was accounted for as the result of linkage between acetyl group and susceptible site of enzyme.

The present study aims at the elucidation of the following points: the inhibitory action of chlorophyllin on proteolytic activity of trypsin, the factors by which the action of the pigment is affected, the recovery of the inert enzyme, and the comparison of the effect of divalent cations on tryptic activity and that of the metal derivatives of chlorophyllin. Moreover, evidences are also presented for the formation of complex between chlorophyllin and trypsin. Besides, interactions between some effective agents and trypsin have been investigated in relation to the mechanism of action to the enzyme. From these experimental facts, a new biological role of metallo-porphyrin compounds will be indicated.

Materials and Methods

Crystalline trypsin used in this study was supplied by Mochida Pharmaceutical Co. (Tokyo), which was prepared according to the method of Northrop¹⁷⁾ and lyophilized. This was carefully dialyzed against 0.001 N hydrochloric acid and made salt-free before use.

Green⁴⁸⁾ pointed out that the protein substrates, Michaelis-Menten constants of which were much larger than the dissociation constants of the inhibitor-trypsin-complexes, were not suitable for the study of competition. Since casein was shown to have comparatively small Michaelis-Menten constant, casein according to Hammarsten was used as material throughout in place of synthetic substrate.

Copper-chlorophyllin (Na-salt) and magnesium-chlorophyllin (K-salt) were supplied by Wako Pure Chemicals, Ltd. and Nippon Biochemical Industries Ltd., respectively, and sodium-salt of iron-chlorophyllin was provided by Dr. E. Yakushiji. These compounds together with other chemicals used in this experiment were purified by recrystallizations before use.

Ovomucoid fraction was prepared from egg-white according to the following procedure: 100 ml. of homogenate of fresh egg-white were precipitated by the addition of 300 ml. cold acetone and filtered. The dried solid cake was extracted with 100 ml. glacial acetic acid by shaking for one hour at room temperature. The filtered extract was added with 300 ml. absolute ethanol, whereby the mucoid fraction separated in flocculent form. This was collected by centrifugation, dissolved in 30 ml. of water, and was dialyzed thoroughly against running water through cellophane membrane (16°, 24 hrs.). The dialysate was, after filtration, diluted up to 100 ml. with distilled water.

Oleate used in this study was prepared by dissolving 28.2 mg. of oleic acid (10^{-4} M) together with small amount of 0.1 N sodium hydroxide and adjusted to pH 7.1 with 0.1 N hydrochloric acid. The volume was also adjusted to 28.2 ml. with distilled water.

Saponifiable fraction of tubercle bacilli was extracted from the three-week cultures of *Mycobacterium tuberculosis** with ether using Soxhlet's apparatus. The extract was saponified with 0.1 N sodium hydroxide, and filtered free from unsaponifiable precipitate. Then, the pH was adjusted to 7.1 with 0.1 N hydrochloric acid. This final fraction amounted to 10 ml. from every 5 g. of wet culture.

In order to extract the saponifiable fraction of tuberculous lesions of experimental rabbit lungs, 24 g. of fresh tuberculous lesions, which were obtained from the albino rabbits infected with 0.1 mg. of two-week culture of *Mycobacterium tuberculosis*, Ravenell strain, at the fourth month from infection, were added with 96 ml. of 1/50 M phosphate buffer, pH 7.1, and homogenized. The homogenate was, then, added drop by drop with 360 ml. of cold acetone. After filtration, the organic solvent was distilled

* Aoyama and BCG strains, both grown on Sauton's medium.

off *in vacuo*, and the resultant insoluble matters were taken up in 24 ml. of cold ether, and saponified with sodium hydroxide. The soap solution was adjusted to pH 7.1, filtered clearly, and was added with distilled water up to 120 ml..

Kunitz's method⁵⁶⁾ for the assay of tryptic activity was slightly modified into two systems as follows: The first assay system (designated as A.S. I):

62.5 H.U.M.*/ml. trypsin solution in phosphate buffer (1/120 M, pH 7.1).....1 ml.

1 mg./ml. solution of sodium-copper-chlorophyllin in phosphate buffer

(1/120 M, pH 7.1).....1 ml.

By the addition of 1/120 M phosphate buffer (pH 7.1), total volume was adjusted to 8 ml..

0.7% solution of casein in phosphate buffer (1/20 M, pH 7.6).....5 ml.

The above mixture except casein was pre-incubated at 30° for 30 minutes, and substrate was then added under cooling. The solution was incubated at 30°, for further 20 minutes, and 5 ml. of the trichloroacetic acid reagent** was added in order to block the reaction and remove unaffected protein. The total volume of A.S. I was 8 ml., and that of the second (A.S. II) was 10 ml., and in these two systems final concentrations were same as regards individual components. Prior to comparative examination of assay systems, they were adjusted so as to contain the same kinds and amounts of the components, and allowed to stand for 30 minutes before filtration. An increase in amount of products of tryptic hydrolysis was measured by an increase in optical density at 280 m μ on acid-soluble fraction by means of spectrophotometer (Hitachi, EPU-2). In some cases, the total non-protein nitrogen was, in part, determined by colorimetric method modified by the present writer³²⁾.

The effect of effective agents on tryptic activity was evaluated by the following value, E or ΔA .

$$E = \Delta A - \Delta B, \text{ and } \Delta A = A_{30} - A_0, \Delta B = B_{30} - B_0,$$

where E represents an effectiveness of the agent, and A is the photometer readings at 280 m μ obtained from the reaction mixture containing effective agent from the first, while B is those from the mixture in which the effective agent was added after blocking the reaction. The subscript such as 30 or 0 denotes experimental temperature. ΔA represents the degree of proteolysis affected by the effective agents, and the ΔB shows that of non-affected proteolysis. Hence, positive E means the acceleration, and negative E the inhibition of enzymatic hydrolysis.

For the investigation of irradiation effect, samples were placed in 10 mm quartz cuvette arranged in a semi-circle around a mercury lamp (Matsuda, 100 KL) at the distance of 10 cm from the surface of lamp under protection against rise of temperature by an electric fan. The whole experiment was carried out in dark room at 15° ± 2° throughout.

*H.U.M. represents a unit of trypsin corresponding to 1/181000 of Anson's hemoglobin unit⁵⁷⁾. 1 H.U.M. of the sample used corresponds to 0.8 mcg of crystalline enzyme.

**Mixture of the equal volumes of aqueous trichloroacetic acid (6.36 g./dl.), sodium acetate (10.6 g./dl.), and glacial acetic acid (7.02 g./dl.), respectively.

Results

1) Anti-tryptic action of chlorophyllins:—Table 1 shows the rate of digestion by trypsin in the presence of sodium-copper-chlorophyllin, sodium-iron-chlorophyllin and potassium-magnesium chlorophyllin at various concentrations ranging from $1.69 \cdot 10^{-6}$ M to $1.00 \cdot 10^{-1}$ M. It is clear that tryptic activity was inhibited by these chlorophyllin compounds, and considerable differences in inhibitory action existed between magnesium derivative and the others. Similar effect was observed both in copper- and iron-derivatives nearly at the final concentration of $4.11 \cdot 10^{-4}$ M, but in lower concentrations copper-derivative more or less exceeded iron-compound, and magnesium-derivative was least active in inhibitory action.

Table 1. Anti-tryptic action of copper, iron and magnesium ions and respective metal derivatives of chlorophyllin. (estimated by A. S. I).

$*E = \Delta A - \Delta B$, where, $\Delta A = A_{30} - A_0$, $\Delta B = B_{30} - B_0$ (Cf. the text.).

Final concentration	E^*					
	Copper		Iron		Magnesium	
	free	combined	free	combined	free	combined
$1.00 \cdot 10^{-1}$ M		-0.306				
$3.33 \cdot 10^{-2}$ M		-0.324				
$1.11 \cdot 10^{-2}$ M		-0.349				
$3.70 \cdot 10^{-3}$ M		-0.357				
$1.23 \cdot 10^{-3}$ M	-0.146	-0.346	-0.039	-0.350	+0.020	-0.296
$4.11 \cdot 10^{-4}$ M	-0.020	-0.338	-0.014	-0.337	+0.016	-0.210
$1.37 \cdot 10^{-4}$ M	+0.018	-0.334	+0.016	-0.282	+0.010	-0.170
$4.57 \cdot 10^{-5}$ M		-0.232		-0.056		-0.013
$1.52 \cdot 10^{-5}$ M		-0.041		-0.029		-0.004
$5.08 \cdot 10^{-6}$ M		-0.004		-0.004		-0.002
$1.69 \cdot 10^{-6}$ M		-0.001		-0.001		-0.001
0		0		0		0

2) Comparison of anti-tryptic action of chlorophyllin at different pH:—The effect of pigments on tryptic digestion within the range of pH 5.0 to 8.5 are depicted in Fig. 1. This curve corresponded to the pH-activity curve of trypsin. The nearer the pH of reaction systems to the optimum pH of tryptic activity, the greater was an inhibition caused by chlorophyllin.

3) Influence of buffer solutions on anti-tryptic action of chlorophyllins:—According to

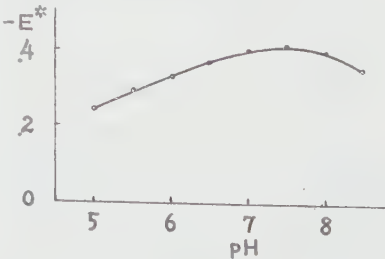


Fig. 1. Relation between pH value and effect of chlorophyllin on tryptic activity. (measured by A. S. I)

* Cf. the text.

Table 2. Relations between various buffer solutions and anti-tryptic action of chlorophyllin (measured by A. S. I).

* Cf. the text.

Final concentration	-E*					
	Buffer					NaCl
	Phosphate	Veronal	Acetate	Borate	Acetate-Citrate	
1/15 M	0.544	0.352	0.675	0.441	0.666	0.706
1/120 M	0.600	0.440	0.605	0.449	0.426	0.612
1/960 M	0.562	0.211	0.364	0.479	0.042	0.338
dist. water	0.280	0.280	0.280	0.280	0.280	0.280

McCann⁵⁸⁾, the salt concentration exerts a significant influence to the effect of blood plasma trypsin inhibitor. Accordingly, it is necessary to examine the effect of buffer solution in detail. From the results obtained, it seemed likely that an optimum concentration of buffer solution existed, at which chlorophyllin showed the maximum inhibition on trypsin. From this viewpoint, buffer solutions used in this experiment could be classified into three groups as shown in Table 2: (1) the optimum concentration stood at the final concentration of 1/120:M, and at higher or lower concentration an appreciable diminution of anti-tryptic activity of chlorophyllin followed. (2) the optimum in question stood at 1/960M, and (3) the optimum concentration stood at 1/15 M. In the last group, a parallelism certainly existed between the concentration of buffer solution and the inhibitory rate of proteolysis by chlorophyllin.

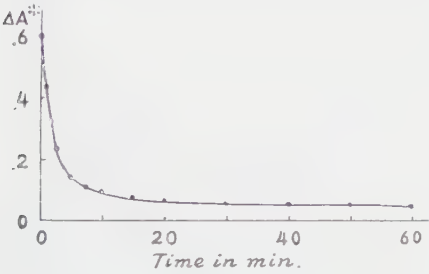


Fig. 2. Relation between pre-incubation and activity of chlorophyllin-trypsin system. (measured by A. S. I)

* Cf. the text.

4) Influence of pre-incubation upon trypsin-inhibition by chlorophyllin:— Fig. 2 shows that the pre-incubation of assay system comprised a key point in anti-tryptic action of chlorophyllin. It is also suggested that chlorophyllin reacted with trypsin according to the first order reaction.

5) Recovery of activity in chlorophyllin-inactivated trypsin.

a) Reactivation by acid-treatment. In an activation experiment of trypsinogen, which

was considered to be an unknown peptide-trypsin compound, by acid treatment, Lisbonne⁵⁹⁾ demonstrated that trypsin could be liberated from trypsinogen within the limited range of pH, that is, at an isoelectric point of the unknown peptide component. Since chlorophyllin was easily precipitated from the aqueous solution in acid reaction, it was interesting to know whether the acid treatment was favourable for reactivation of trypsin previously inactivated by chlorophyllin. In this experiment, a chlorophyllin-trypsin mixture, which was pre-incubated at 30° for 30 minutes, was carefully added with increasing amount of hydrochloric acid under cooling,

whereby pH was adjusted to various values ranging from 2.00 to 7.09. The mixtures were allowed to stand at 37° for two hours. Substrate was, then, added to the mixture under cooling, and the activity of enzyme was measured as already mentioned. As can be seen from Fig. 3, the enzyme once blocked seemed to be activated again in an acid reaction (nearly at pH 4.2), but not in neutral reaction.

b) Reactivation by means of ammonium sulfate. Jacobsson⁶⁰⁾ reported on the reactivation effect of urea on trypsin affected by soybean inhibitor. According to him, inhibitor which is protein in nature has to be separated and precipitated by urea resulting in a liberation of active trypsin. Ammonium sulfate behaved similarly, and even promoted the catalytic activity of trypsin at lower concentrations as shown in Table. 3. On the other hand, chlorophyllin itself reacted with ammonium sulfate and formed an insoluble complex. Therefore, it was likely that this salt could attenuate the blocking effect of chlorophyllin on proteolysis. In fact, the de-inhibitory action of ammonium sulfate against chlorophyllin-trypsin mixture was ascertained in the present experiment, in which the pre-incubated pigment-enzyme mixture was added with various amounts of ammonium sulfate and incubated at 37° for further two hours. Then, the reaction mixture was treated as usual and the photometer readings at 280 m μ of the resulted acid-soluble fraction are arranged in Table. 3.

6) Effect of free heavy metal-ions on tryptic activity:—From the data in Table 1, it is evident that Cu⁺⁺ and Fe⁺⁺ inhibited the activity of trypsin, but their effects were much smaller than those of Cu- and Fe-derivatives of chlorophyllin at an equal concentration respective

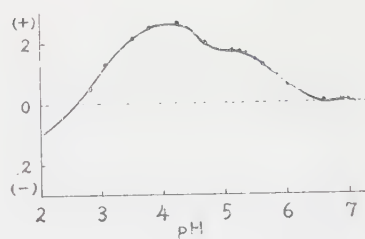


Fig. 3. Reactivation of trypsin affected by chlorophyllin by treatment with 1/50 M HCl. (measured by A. S. II) Ordinate: the difference between ΔA values obtained from the treated and non-treated systems, respectively. The dotted line shows the level of tryptic activity before acid-treatment.

Table 3. Effect of ammonium sulfate on the activity of trypsin and reactivation of chlorophyllin-trypsin-complex treated with ammonium sulfate at lower concentrations.

(measured by A. S. II). * Cf. the text.

Final concentration	ΔA^*	
	Effect of $(\text{NH}_4)_2\text{SO}_4$ on tryptic activity	Effect of $(\text{NH}_4)_2\text{SO}_4$ on reactivation
1/20 M	0.518	0.580
1/30 M	0.527	0.580
1/35 M	0.587	0.589
1/40 M	0.627	0.607
1/50 M	0.631	0.600
1/60 M	0.661	0.581
1/70 M	0.627	0.580
1/85 M	0.627	0.570
1/110 M	0.616	0.564
1/340 M	0.607	—
dist. water	0.567	0.552

of the metals concerned. Mg^{++} tended to promote proteolysis contrary to the fact that Mg-chlorophyllin acted rather unfavourably against tryptic activity as already mentioned (*cf.* Tab. 1).

7) Effect of potassium cyanide on the anti-tryptic activity of sodium-iron-chlorophyllin:—Potassium cyanide showed almost no inhibitory effect on trypsin at and near neutral reaction as shown in Table 4.

Table 4. Effect of potassium cyanide on inactivation of trypsin by chlorophyllin (estimated by A. S. II). * *Cf.* the text.

No.	Composition of reaction systems				ΔA^*
	Final concentration of chlorophyllin		Final concentration of K C N		
	$5 \cdot 10^{-5}M$	$1 \cdot 10^{-5}M$	$1.2 \cdot 10^{-5}M$	$2.4 \cdot 10^{-5}M$	
1	+				0.490
2	+		+		0.511
3			+		0.720
4	+				0.489
5	+			+	0.490
6				+	0.727
7		+			0.600
8		+		+	0.660
9				+	0.726
10		+			0.639
11		+		+	0.639
12				+	0.738
13					0.744

It might well be assumed that the iron-chlorophyllin reacted with potassium cyanide in similar fashion as hemin did. Therefore, a mixture of iron-chlorophyllin and potassium cyanide was incubated at 30° for 30 minutes, and trypsin was, then, added to the mixture and kept again at 30° for 20 minutes. The rate of proteolysis was estimated thereafter. The results were summarized in Table 4. It became evident that an unfavourable effect of iron-chlorophyllin on proteolysis could be abolished by added potassium cyanide.

8) Absorption coefficients and dissociation constants of chlorophyllins:—Chlorophyllins intensely absorb light at three wave lengths (Fig. 4), and the absorption coefficients of copper derivative in 1/15 M borate buffer (pH 7.1, 20°) stood at $\epsilon_{236}=2.64 \cdot 10^5$, $\epsilon_{497}=3.52 \cdot 10^5$ and $\epsilon_{630}=9.6 \cdot 10^6$, respectively.

For the determination of dissociation constants (pKa') of chlorophyllins, pigments were

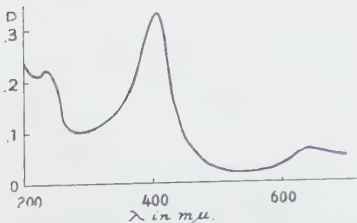


Fig. 4. Absorption spectrum of Cu-chlorophyllin.

dissolved in buffer solutions having various pH values ranging from 1.0 to 11.4, and the optical densities at the Soret band were measured. From the curves in Fig. 5, pK_a' values of chlorophyllins were obtained as follows:

Copper-chlorophyllin: pK_{a1}' ; 3.7, pK_{a2}' ; 8.2

Iron-chlorophyllin: pK_{a1}' ; 3.7, pK_{a2}' ; 8.7

Magnesium-chlorophyllin: pK_{a1}' ; 3.7, pK_{a2}' ; 7.6

9) Absorption spectrum of the mixture of chlorophyllin and trypsin:—The spectrochemical study on this mixture was carried out with the following three samples; (1) the phosphate buffer solution of trypsin (pH 8.0), (2) the phosphate buffer solution of sodium-iron-chlorophyllin (pH 8.0), and (3) a mixture of equal volumes of (1) and (2). The solutions were treated at 30° for 30 minutes before measurements. The results showed that the Soret band at 407 $m\mu$ of sodium iron chlorophyllin shifted onto 410 $m\mu$ and simultaneous exaltation in optical density occurred after mixing both components (Fig. 6).

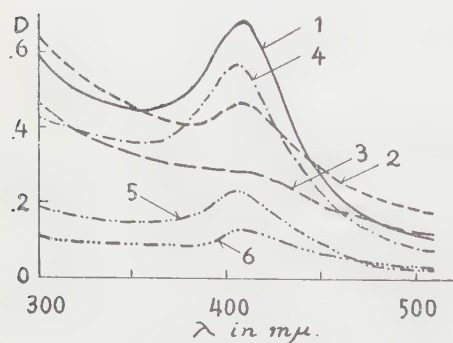


Fig. 6. Absorption spectra of chlorophyllins and of the mixtures of trypsin and pigment. The measurement was made after keeping at 30° for 30 minutes. Curves 4, 5 and 6 show the absorption spectra of various concentrations of Fe-chlorophyllin, and 1, 2 and 3 show those of the corresponding chlorophyllin-trypsin mixtures. The contents of the components were 5 mcg./ml. of chlorophyllin for curves 1 and 4, 2 mcg./ml. for curves 2 and 5, 1 mcg./ml. for curves 3 and 6, and 6 mg./ml. of trypsin for curves 1, 2 and 3.

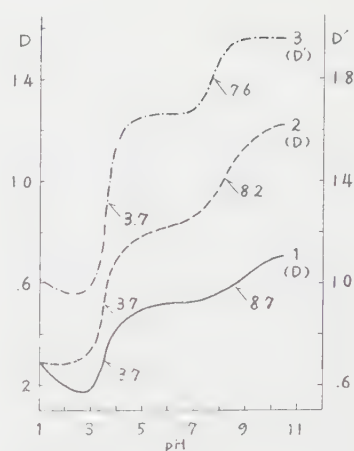


Fig. 5. Dissociation curves of Fe- (1), Cu- (2), and Mg (3) derivatives of chlorophyllin.

10) Absorption spectrum of the mixture of chlorophyllin and histidine or trypsin in the presence of potassium cyanide:—An attempt was made to study the effect of potassium cyanide on the formation of chlorophyllin-trypsin complex. In this experiment, a phosphate buffer solution (pH 8.0) of iron-chlorophyllin was mixed with potassium cyanide solution, and this mixture was added to the phosphate buffer solution (pH 8.0) containing histidine which was considered by Gutfreund⁽⁶¹⁾ to be an essential constituent of trypsin for its catalytic action. Apparently, a considerable difference was observed between the spectrum of chlorophyllin-trypsin mixture and that of the pigment-potassium cyanide-trypsin mixture, as shown in Fig. 7. Similar results were also obtained with trypsin in place of histidine.

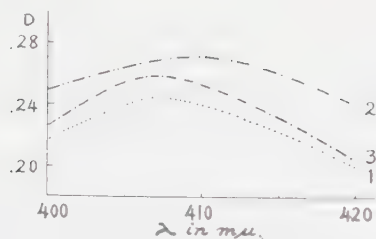


Fig. 7. Absorption spectra of chlorophyllin-KCN mixture (1), chlorophyllin-histidine mixture (2) and chlorophyllin-KCN-histidine mixture (3). Final concentration of each component: histidine 0.97 mg./ml.; KCN 1/300 M; Fe-chlorophyllin 1/20000 M; each in phosphate buffer, pH 7.5.

11) Absorption spectrum of the mixture of chlorophyllin and casein:—Light absorption of the chlorophyllin-casein mixture was measured in the same way as above. It was found that the spectral curve of the mixture lay just in a half way between the curves of the two single components, as shown in Fig. 8.

12) Dissociation constant (K_i) of chlorophyllin-trypsin complex and Michaelis-Menten constant (K_m) of trypsin-casein system:—1/20 M phosphate buffer solutions (pH 7.1) containing casein in varying concentrations were added with trypsin together with chlorophyllin in the cold, and the solution was stood at 30° during 10 minutes. After fixing the reaction with trichloroacetic acid, the optical density of an acid-soluble fraction was measured as already mentioned, and the

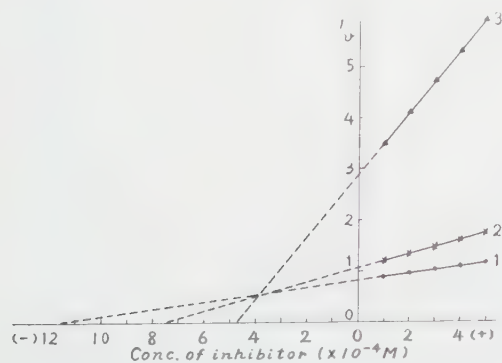


Fig. 9. Estimation of dissociation constant of chlorophyllin-trypsin compound. The concentration of substrate corresponds $3.2 \cdot 10^{-5}$ M to curve 1, $1.6 \cdot 10^{-5}$ M to curve 2 and $3.2 \cdot 10^{-6}$ M to curve 3, respectively.

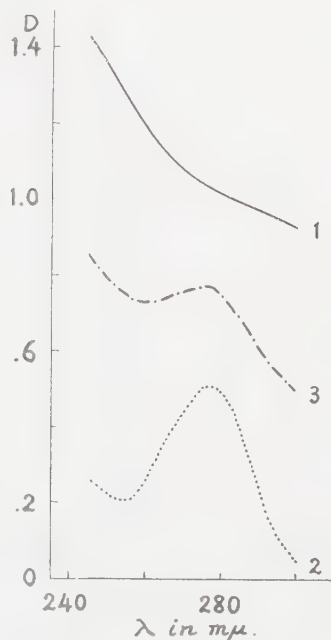


Fig. 8. Absorption spectra of chlorophyllin (1), casein (2) and 1:1 mixture of both (3). The concentration was 30 mcg/ml for chlorophyllin, and 0.07% for casein. Phosphate buffer, pH 8.0, was used as solvent.

values obtained were plotted according to Dixon's method⁶². The following results were obtained therefrom: about $3.68 \cdot 10^{-4}$ M for K_i and $1.48 \cdot 10^{-5}$ M for apparent K_m . But the molecular weight of casein used in this study was assumed as 89,000. As seen from Fig. 9, three linear curves obtained cut each other at a point not on the base line. Here, it must be added that in a range of concentrations of substrate higher than $6.4 \cdot 10^{-6}$ M, linear curves were not obtained at any concentrations of chlorophyllin by Lineweaver-Burk's plotting method⁶³.

Table 5. Effect of calcium chloride on the activity of trypsin.
* Cf. the text.
(measured by A. S. I)

Final concentration	ΔA^*
1/12.5 M	0.447
1/6.25 M	0.626
1/3.13 M	0.726
1/1.56 M	0.767
1/0.78 M	0.727
dist. water	0.707

chloride, the photometer readings at 280m μ of acid-soluble fractions were larger in calcium-containing system than in the others, and it seemed that the inhibitory action of chlorophyllin was abolished by calcium chloride. This tendency was more or less disturbed in the systems pre-incubated with substrate but not with enzyme (see Tab. 6). b) Diluted ammonium sulfate, too, accelerated the proteolytic activity of trypsin as shown in Table 3. From the same interaction test as above, the similar results were obtained in the case of this salt, as shown in Table 7.

c) Tables 8 and 9 show that ovomucoid inhibited the catalytic hydrolysis of casein, but an additional effect was brought about by chlorophyllin. Therefore, an inhibitory effect of chlorophyllin on trypsin seemed to be reduced by added ovomucoid. An additional effect could, however, be recognized in the system containing all components prior to reaction.

d) Oleate showed an inhibitory effect on trypsin (see Tab. 10). In the presence of both chlorophyllin and oleate, the pre-incubated reaction systems gave smaller photometer-readings at 280 m μ than without oleate. The effects of oleate and chlorophyllin seemed to be additional (see Tab. 11).

e) The saponifiable fractions of both virulent and avirulent

13) Interaction between chlorophyllin and some agents, which were favourable for trypsin activity:— a) The present writer examined the effect of calcium chloride on the activity of trypsin and found that there was an optimum concentration for the promotion of tryptic activity as shown in Table 5. For the test of interaction between pigment and calcium chloride, individual components of assay system were successively added in different order. Among the pre-incubated systems in the presence of both enzyme and chlorophyllin and/or calcium

Table 6. Effect of chlorophyllin on the activity of trypsin affected by calcium chloride.
Values of ΔA were measured by A. S. II.
* Cf. the text. E: trypsin, S: casein, C: chlorophyllin, X: CaCl₂, at final concentration of 1/1.56 M

Pre-incubation (30°C, 30 min.)	Reaction (30°C, 20 min.)		ΔA^*
EX	S	C	0.601
EX	SC		0.505
ECX	S		0.435
EC	SX		0.050
EC	S	X	0.047
SX	E	C	0.625
SC	EX		0.581
CX	ES		0.545
SX	EC		0.530
SCX	E		0.515
SC	E	X	0.447
	ESX	C	0.774
	ES	CX	0.544
	ESCX		0.510
	ESC	X	0.435

Table 7. Effect of chlorophyllin on the activity of trypsin affected by ammonium sulfate. (measured by A. S. II)

* Cf. the text. E: Trypsin, S: casein, C: chlorophyllin, X: $(\text{NH}_4)_2\text{SO}_4$, at final concentration of 1/60 M

Pre-incubation (30°, 30 min.)	Reaction (30°, 20 min.)		JA ¹
EX	S	C	0.777
EX	SC		0.727
ECS	S		0.677
EC	SX	X	0.198
EC	S		0.169
SX	E	C	0.917
SC	E		0.837
SCX	E		0.834
SX	EC	X	0.834
CX	ES		0.834
SC	E		0.834
	ESX	C	0.794
	ES	CX	0.692
	ESCX		0.663
	ESC	X	0.526

Table 9. Effect of chlorophyllin on the activity of trypsin affected by ovomucoid fraction. (measured by A. S. II)

* Cf. the text. E: trypsin, S: casein, C: chlorophyllin, X: ovomucoid, at final concentration of 1: 800

Pre-incubation (30°, 30 min.)	Reaction (20°, 20 min.)		JA*
EX	S	C	0.311
EX	SC		0.289
ECX	S		0.079
EC	S	X	0.069
EC	SX		0.046
SX	E	C	0.602
SCX	E		0.391
SC	E		0.383
SC	EX	X	0.381
SX	EC		0.381
CX	ES		0.292
	ES	CX	0.605
	ESX	C	0.361
	ESC	X	0.351
	ESCX		0.212

Table 8. Effect of ovomucoid fraction on activity of trypsin. (measured by A. S. I)

* Cf. the text.

Final concentration	JA ¹
1: 200	0.127
1: 300	0.148
1: 400	0.161
1: 800	0.226
1: 2000	0.386
1: 3000	0.517
1: 4000	0.627
1: 8000	0.713
dist. water	0.817

Table 10. Effect of oleate on the activity of trypsin.

Each value was measured by A. S. I.

* Cf. the text.

Final Concentration	JA*
1/ 100 M	0.206
1/ 200 M	0.207
1/ 400 M	0.207
1/ 600 M	0.236
1/ 800 M	0.266
1/ 3300 M	0.298
1/ 5000 M	0.341
1/10000 M	0.447
1/20000 M	0.520
1/40000 M	0.577
1/80000 M	0.626
dist. water	0.722

strains of *Mycobacterium tuberculosis* were tested in connection with the blocking effect of chlorophyllin on trypsin. These fractions brought forth a considerable inhibition toward the proteolytic activity of trypsin as shown in Tables 12, 13 and 14.

The interaction between the pigment and the above fractions was not, however, essentially different in comparison with the case of oleate, except that the actions of chlorophyllin and tubercle fractions were weakened each other in the systems pre-incubated. In these experiments, the rate of proteolysis was represented by an increase of total nitrogen of acid-soluble fractions by an improved method of colorimetry³²⁾.

f) An extract from the tuberculous lesions of experimental rabbit lungs was also shown to have an unfavourable effect on trypsin (see Tab. 15). So, an experiment was made to settle the question: is there any interaction between the lung-extract and chlorophyllin? The result was found to be almost the same as that of the extracts of tubercle bacilli (see Tab. 16). The activities of enzyme were determined by the measure of total nitrogen contained in acid-soluble fractions.

14) Effect of chlorophyllin on autolysis of trypsin:—Fig. 10 shows that, within 4-hour period

Table 11. Effect of oleate on the activity of trypsin. (measured by A. S. II)
* Cf. the text. E: trypsin, S: casein, C: chlorophyllin, X: oleate, at final concentration of 1/5000 M

Pre-incubation (30°, 30 min)		Reaction (30°, 20 min.)		ΔA*
EX	S	C		0.368
EX	SC			0.349
EC	SX			0.093
EC	S	X		0.074
ECX	S			0.065
SX	E	C		0.633
SC	EX			0.633
SC	E	X		0.619
SX	EC			0.605
SCX	E			0.587
CX	ES			0.555
	ES	CX		0.633
	ESX	C		0.633
	ESC	X		0.535
	ESCX			0.503

Table 12. Effect of saponifiable fraction of tubercle bacilli on trypsin-activity.
* Cf. the text.

Final concentration	ΔA*	
	Fraction from virulent strain	avirulent strain
1: 80	0.529	0.569
1: 500	0.520	0.537
1: 750	0.500	0.485
1: 1000	0.496	0.477
1: 2500	0.498	0.507
1: 3750	0.516	0.526
1: 5000	0.522	0.524
dist. water	0.525	0.525

after initiation of autolysis, no increase in optical density was brought about by an acid-soluble fraction of the chlorophyllin-containing system, although the optical density increased appreciably by the same fraction obtained from chlorophyllin lacking one. At the end of 24-hour period, trypsin commenced to undergo autolysis in both cases. At this stage, difference in photometer-readings at 280 mμ between

Table 13. Effect of chlorophyllin on the activity of trypsin affected by saponifiable fraction from virulent strain of *M. tuberculosis*. (measured by A. S. II)

* Cf. the text. E: trypsin, S: casein, C: chlorophyllin, X: saponified fraction, at final concentration of 1: 1000.

Pre-incubation (30', 30 min.)	Reaction (30', 20 min.)		J.I.*
EX	S	C	0.467
EX	SC		0.367
ECX	S		0.141
EC	S	X	0.138
EC	SX		0.123
SC	E	X	0.603
SX	E	C	0.577
SCX	E		0.571
SC	EX		0.551
SX	EC		0.531
CX	ES		0.484
	ES	CX	0.581
	ESX	C	0.577
	ESC	X	0.476
	ESCX		0.457

Table 14. Effect of chlorophyllin on the activity of trypsin affected by saponified fraction from avirulent strain of *M. tuberculosis*. (measured by A. S. II)

* Cf. the text. E: trypsin, S: casein, C: chlorophyllin, X: saponified fraction, at final concentration of 1: 1000

Pre-incubation (30', 30 min.)	Reaction (30', 20 min.)		J.I.*
EX	S	C	0.416
EX	SC		0.374
ECX	S		0.147
EC	S	X	0.136
EC	SX		0.136
SX	E	C	0.621
SCX	E		0.581
SC	E	X	0.572
SC	EX		0.567
SX	EC		0.547
CX	ES		0.487
	ES	CX	0.583
	ESX	C	0.563
	ESC	X	0.517
	ESCX		0.477

Table 15. Effect of saponifiable fraction of tubercles on tryptic activity. (measured by A. S. I)

* Cf. the text.

Final concentration	J. A*
1: 8	0.509
1: 16	0.588
dist. water	0.638

the two systems was found to be almost the same as that measured at the end of the first 4-hour period.

15) Effect of chlorophyllin on UV-denaturation of trypsin:—

As reported by Pace⁵²⁾ and other authors⁵³⁾, trypsin was denatured by ultraviolet irradiation, as shown in Fig. 11. Denaturation curves were made by plotting the difference of the photometer-readings at 280 m μ obtained from the irradiation and non-irradiation systems. In chlorophyllin-free systems, the difference in optical density at 280 m μ increased almost in parallel with the irradiation time. But, in chlorophyllin-containing systems, the difference increased rapidly at an early stage of irradiation, but slowly and slightly at later stages.

16) Effect of chlorophyllin on heat-denaturation of trypsin:—

a) For the present purpose, the denaturation was indicated

Table 16. Effect of chlorophyllin on the activity of trypsin affected by saponified fraction from tuberculous lesions of experimental rabbit lungs. (Each value was obtained by A. S. II).

*Cf. the text. E: trypsin, S: casein, C: chlorophyllin, X: saponified fraction, at final concentration of 1: 8.

Pre-incubation (30°, 30 min.)	Reaction (30°, 20 min.)	ΔA^*
EX	S	C 0.569
EX	SC	0.547
ECX	S	0.471
EC	S	X 0.158
EC	SX	0.155
SC	E	X 0.671
SX	E	C 0.613
SC	EX	0.589
SCX	E	0.577
CX	ES	0.573
SX	EC	0.571
	ES	CX 0.667
	ESX	C 0.607
	ESC	X 0.586
	ESCX	0.557

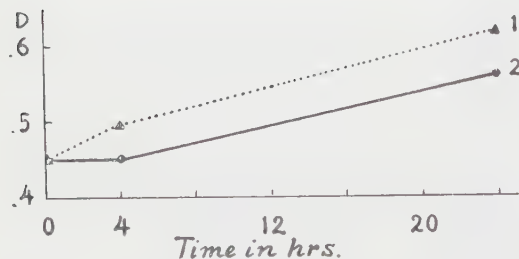


Fig. 10. Change in content of acid-soluble fractions during the course of autolysis of trypsin. 1: system without the pigment, 2: system with the pigment. Trypsin: 5 mg./ml. in 1/15 M phosphate buffer, pH 7.1; total volume: 20 ml. ordinate: increase in photometer-readings at 280 μ .

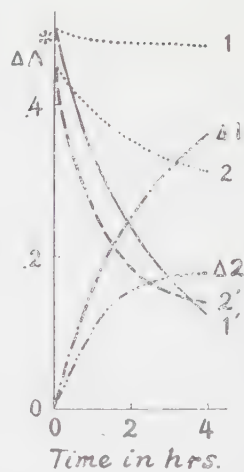


Fig. 11. Effect of chlorophyllin on denaturation of trypsin by UV irradiation. Cf. the text. Curves 1 and 1' show the changes in photometer-readings at 280 $m\mu$ in chlorophyllin-free systems, and curves 2 and 2' show those in pigment-containing systems. Curves 1 and 2 are related to non-irradiated systems, and 1' and 2' to the irradiated systems. $\Delta 1 = 1 - 1'$, $\Delta 2 = 2 - 2'$; trypsin: 240 H.U.M./ml.; chlorophyllin: 1 mg./ml.; solvent: 0.14 M NaCl.

by the rate of proteolysis by means of the systems pre-incubated at 30° or 50°. As shown in Fig. 12, enzyme activity was lost rapidly within the first 30 minutes in pigment-containing system, and the curve ran almost parallel with abscissa. In chlorophyllin-free system, the enzyme activity decreased only slightly on pre-incubation at 30°, while in the case of pre-incubation at 50°, significant but gradual loss of activity was observed throughout the whole range of

incubation time tested. Accordingly, two curves, which were made by plotting the difference of the photometer-readings at 280 m μ obtained from the preincubated systems at 30° or 50°, corresponding to the presence or absence of chlorophyllin, were separated from each other, according to the length of preincubation time.

b) The change in tryptic activity was carefully examined by heating the neutral enzyme solution at 92° for 10 minutes. Table 17 shows that in systems having no chlorophyllin, the inert trypsin affected by heat was slightly reactivated after standing for two hours within the temperature range 0°~20°, but not the case in chlorophyllin-containing system. When the substrate was added prior to heating, the reactivation occurred to the same extent, irrespective of the presence or absence of chlorophyllin (Tab. 17).

c) Following tests were also carried out: pre-incubated systems were added with a

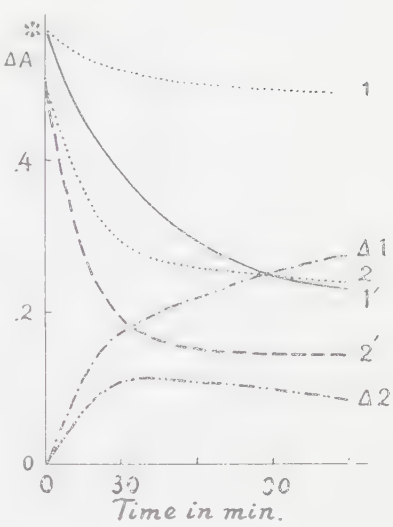


Fig. 12. Effect of chlorophyllin on the denaturation of trypsin by heat. * Cf. the text. Curves 1 and 1' show the changes in photometer-readings at 280 m μ in the chlorophyllin-free systems and 2 and 2' show those in the pigment-containing systems. Curves 1 and 2 were obtained with non-heated systems and 1' and 2' with heated ones. $\Delta 1 = 1 - 1'$, $\Delta 2 = 2 - 2'$; trypsin: 240 H. U. M./ml.; chlorophyllin: 1 mg./ml.; reaction medium: phosphate buffer; total volume: 4 ml..

Table 17. Effect of chlorophyllin on the reactivation of heat-denatured trypsin.

PS: Substrate was added after heating the enzyme solution at 92° for 10 minutes, and the system was kept at various temperatures for various length of time. The activity was measured by A. S. II.

AS: Substrate was added before treatment.

* Cf. the text. ** photometer-readings at 280 m μ of acid-soluble fractions.

Trypsin: 240 H. U. M./ml.; chlorophyllin: 1 mg./ml.; solvent: 0.14 M NaCl; total volume: 4 ml..

Condition of cooling after heat-treatment	Chlorophyllin (+)		Chlorophyllin (-)	
	PS (ΔA^*)	AS (D **)	PS (ΔA^*)	AS (D **)
0°, 120 min. \rightarrow 20°, 0 min.	0.000	0.580	0.261	0.610
0°, 90 " \rightarrow 20°, 30 "	0.032	0.760	0.260	0.809
0°, 60 " \rightarrow 20°, 60 "	0.026	0.890	0.281	0.933
0°, 30 " \rightarrow 20°, 90 "	0.041	1.112	0.281	0.992
0°, 0 " \rightarrow 20°, 120 "	0.031	1.192	0.280	1.030

series of 1/15 M buffer solutions (acetate buffers of pH 4.0~4.8, and phosphate buffers of pH 5.2~8.3) and kept at 42° for 12 hours and their enzyme activities were followed at various time intervals. From these results, it was shown that in zero time, the activity of trypsin became higher at higher pH value, however at the second hour of heating, the pH-activity-curve of chlorophyllin-trypsin mixture showed the maximum value at pH 6.0. After 12 hours of incubation, this peak shifts into an alkaline side (pH 7.1~7.6). When a system having no chlorophyllin was kept at 42° for 12 hours, the activities of trypsin fell off in the strong alkaline region (see Fig. 13).

Discussion

Although an appreciable number of researches have been published in relation to the bacteriostatic and/or bactericidal actions of chlorophyllin, according to which the present writer can understand the said effects as the secondary manifestation of its inhibitory actions toward enzyme systems, only a few can be enumerated as regards the incipient effect of the pigment on enzymes, namely the work of Rapp¹¹⁾, Vecchio¹²⁾, Wasielewski *et al.*⁶⁾, and Zirm and his co-workers¹³⁾, the last two investigations of which authors dealt with proteolytic enzymes, but gave contradictory results each other as to the effect of the pigment. This is probably due to the difference of enzyme source on one hand, and to the impurity of materials on the other. Therefore, precise examinations were carried out by the present writer using the purest samples of chlorophyllin derivatives and crystalline trypsin. In fact, an anti-tryptic action was demonstrated in any of the three chlorophyllins even in their lower concentrations. The chlorophyllin derivatives used contain divalent metals, Mg, Fe, and Cu, respectively, and some of these metal ions have been proved to have an inhibitory action against trypsin. It is known that neither Cu nor Fe ion inhibits trypsin in such a concentration that just corresponds to an effective concentration of respective chlorophyllin derivatives, however, Mg ion more or less promotes an enzymatic proteolysis under the same condition. Accordingly, it follows that chlorophyllins themselves have an anti-tryptic capacity, which is different from that of the metal ions alone.

From the pre-incubation-activity curve of chlorophyllin-trypsin system, it is

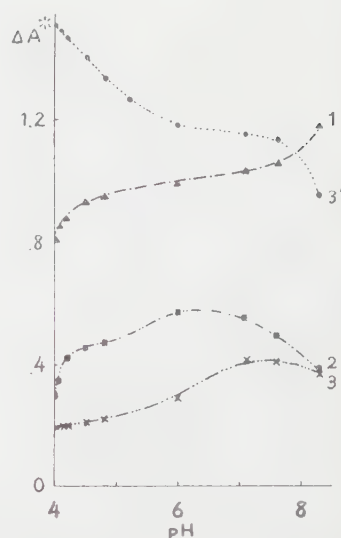


Fig. 13. Change in activity of chlorophyllin-trypsin complex during the course of incubation at 42° in phosphate buffer. * Cf. the text. Curve 1: plotted by zero time readings. 2: after 4-hour incubation. 3: after 12-hour incubation. 3': corresponding to the chlorophyllin-free system after 12-hours. trypsin: 500 H. U. M. ml.; chlorophyllin: 1 mg./ml.; total volume: 4 ml..

indicated that the inhibition proceeds in accordance with the reaction of the first order. Thus, one of the plausible mechanisms of anti-tryptic action of chlorophyllin may be as follows: (1) Chlorophyllin combines with the chlorophyllin-affinic groups of trypsin at and/or near the site of active center of the enzyme according to the first order reaction. (2) Thus, the masking of the enzyme is followed by a loss of proteolytic activity.

The reactivation of chlorophyllin-trypsin complex by acid-treatment seems to be due to its dissociation, since pH-dependence of the reactivation can be recognized. An inert trypsin-chlorophyllin complex can be reactivated also by ammonium sulfate. In this case, the release of chlorophyllin-residues should be brought about by ammonium sulfate resulting in a liberation of active trypsin.

Direct evidence for the formation of chlorophyllin-trypsin complex has been obtained by spectroscopic examinations, in which a shift of absorption at Soret band towards longer wave-length as well as an exaltation in optical density was observed after mixing trypsin with chlorophyllin. As mentioned above, metallo-porphyrin has been shown to combine with other substances, e.g., protein, at the site of its metal and/or side chains. The similarity of the spectrum of Fe-chlorophyllin-cyanide-trypsin complex to that of chlorophyllin itself, and the neutralization of anti-tryptic action of Fe-chlorophyllin by potassium cyanide, strongly support the concept that the iron of Fe-chlorophyllin is involved in the combination between chlorophyllin and enzyme protein. In other metallo-chlorophyllins the central metals, such as Cu and Mg, play surely an essential role in bringing about association complexes, in view of the facts that the metal ions such as Cu, Zn and Cd are capable of forming complexes with histidine, proteins, etc.⁶⁴⁻⁶⁶.

Whatever residues in protein molecule may be occupied by porphyrin-metals, an actual state of protein molecule, as a whole, should be also taken into account, because of the fact that the smaller the protein-molecule becomes, the smaller is the capacity of its linkage-formation. At present, it becomes probable that the histidine residues in protein take part in the linkage between metallo-porphyrin and protein⁶⁴⁻⁶⁶, although the heme-affinity of free histidine is found to be rather small as shown by Horiguchi⁴³. The present writer could demonstrate the formation of association complex between free histidine and chlorophyllin by spectrochemical means. On the other hand, histidine is proved to be an essential component of trypsin⁶¹, so it is highly probable that the linkage of chlorophyllin with trypsin resides in the imidazole ring of histidine in enzyme molecule. The pKa' values of chlorophyllins were found as 3.7 and about 8.0. Among them, the former apparently depend on the dissociation of carboxyl group, and the latter on that of the metal. Wyman³⁶) stated in his study on horse oxyhemoglobin that the imidazole group in histidine residue is active within the range of pH 5.5 to 8.5. On the other hand, Kajita *et al.*³¹) have demonstrated that heme combines with histidine easily in their dissociated state. Therefore, it is surmised that the imidazole ring in trypsin and

the metal in chlorophyllin molecule are readily brought into contact to form co-ordination compound. If this is true, reasonable explanation may be possible for the fact that the optimum pH for the inhibitory action of chlorophyllin lies at about pH 8, as stated above.

According to Ram *et al.*⁵⁴⁾ the pancreas inhibitor for trypsin combines with tryptophane or tyrosine residue in trypsin, however, ovomucoid and soybean inhibitors for trypsin combine with some other residues in the enzyme. As regards the combination between chlorophyllin and trypsin, tryptophane or tyrosine residue seems, however, to be excluded, because chlorophyllin does not combine with tryptophane or tyrosine residue of casein, as mentioned above.

The inhibition followed by the complex formation should be regarded as an usual type of competition from the fact that the Dixon's relation holds good between reciprocal velocity and concentration of inhibitor and the resultant three lines intersect at a point not on the base line. But, the inhibitory action of chlorophyllin is probably different from that of protein inhibitors in a manner just pointed out by Green⁴⁸⁾. As already provided, the curves obtained by Lineweaver-Burk's method do not run in linear fashion at higher concentrations of the substrate. This indicates the choking of active sites by means of substrate molecules.

The release of anti-tryptic action of chlorophyllin is brought about by ammonium sulfate, calcium chloride and hydrochloric acid. In spite of the similarity in their final effect, these substances may be different from each other in their mode of action, since chlorophyllin forms an insoluble precipitate with ammonium sulfate or hydrochloric acid but not with calcium chloride.

From the estimation of proteolytic activity affected by some known- and unknown-inhibitors in the presence of chlorophyllin, it is revealed that in the presence of inhibitors, the release of inhibition by chlorophyllin takes place irrespective of pre-incubation of the reaction system. In an inhibitor such as oleate, effect is only additive to that of chlorophyllin in the absence of pre-incubation, but, otherwise, the effect of the pigment results only in decrement. Such different modes of interaction may be ascribed in part to the presence of a number of active sites in trypsin molecule, as recently demonstrated in the case of pepsin⁵⁵⁾. Each of these active sites in trypsin shares its proteolytic activity, and the inhibitor molecules occupy some of these active sites in the enzyme molecules, resulting in the formation of various inhibition types. Similar view has been opened by Nagahisa⁶⁷⁾ in his studies on the effect of ultraviolet irradiation on tryptic activity.

Now, chlorophyllin shows an anti-autolytic action to trypsin molecule especially at an early stage of incubation, but this effect considerably decreases on prolonged incubation. This may be explained as follows: At first, chlorophyllin combines with chlorophyllin-affinic groups of trypsin, and masks their active centres, which are, however, gradually unmasked in later stages of incubation, probably on account of advanced loosening or unfolding of trypsin molecule. Thus, trypsin regains its

capacity of autolysis. In this connection, it is also recognized that the autolysis is pH-dependent, namely, in the presence of chlorophyllin, the lower the pH-level becomes, the more the autolysis goes on. The relation is just reverse in the absence of the pigment.

As regards the probable role of chlorophyllin on UV-denaturation of trypsin, the following three possibilities might be taken into consideration: (1) protection from UV-damage as a light-filter, (2) photo-sensitization for denaturation of enzyme protein and (3) stabilization of enzyme protein. However, the first point is less probable because of the fact that a considerable difference in light absorption exists between enzyme and chlorophyllin in a spectral region of shorter wave lengths, and, moreover, the quantum yield in UV-denaturation of enzyme is very small⁽⁶⁸⁾. Second, the "apparent" photo-sensitization for denaturation of enzyme protein as observed in Fig. 11 seems to be due primarily to an acceleration of reversible complex formation between chlorophyllin and trypsin. Accordingly, the third point appears to be most reasonable, because the denaturation curves obtained from chlorophyllin-containing reaction systems (J2 in Fig. 11) run below in comparison with those from pigment-free systems (J1 in Fig. 11).

Summary

1. Copper-, iron- and magnesium-derivatives of chlorophyllin were investigated in relation to their effects on crystalline trypsin, and it was ascertained that these derivatives inhibited the proteolytic activity of trypsin.

2. The inhibition of chlorophyllins toward trypsin was greater than that of the corresponding component-metals alone, so that the inhibitory action of chlorophyllin had to be ascribed to the effect of chlorophyllin itself.

3. Pre-incubation constituted a key point in the study of inhibitory action of chlorophyllin toward trypsin, and the pre-incubation-activity curve indicated the reaction of the first order, from which one possible mechanism of inhibitory action of chlorophyllin was deduced.

4. By spectrochemical studies on chlorophyllin-trypsin mixtures, the formation of complex compound was demonstrated.

5. Trypsin was affected by chlorophyllin to form inert complex compound, which could be reactivated by treatment with acid or ammonium sulfate.

6. The combination between chlorophyllin and trypsin took place at the sites: iron atom in chlorophyllin on one hand, and imidazole ring of histidine residue in trypsin on the other.

7. The mode of action of chlorophyllin toward trypsin was of usual type of competition.

8. The inhibitory action of chlorophyllin was nullified by some accelerators for trypsin such as CaCl_2 and $(\text{NH}_4)_2\text{SO}_4$.

9. The anti-tryptic activity of chlorophyllin was weakened by some trypsin-

inhibitors under certain experimental conditions. In some cases, the effect of chlorophyllin was shown to be additive in the concomitance of other inhibitors.

10. Chlorophyllin was rather protective against the autolysis of enzyme protein.

11. It seemed very probable that chlorophyllin played a role as a stabilizer for trypsin molecule in UV-irradiation.

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摘 要

トリプシンは、 α -ナフチル-L-グルタミン酸を基質として、Wasielowski 等、および Zirm 等の報告が見られるが、両者の結果は一致していない。このことは用いられた酵素源のちがうこと、純化されていない資料を用いたことによると思われる。筆者は結晶トリプシン、および 3 種の結晶クロロフィリン、クロロフィリン *a*、クロロフィリン *b*、クロロフィリン *c* の影響を調べた。その結果：① Cu-, Fe- および Mg-クロロフィリンはいずれもトリプシンの作用を阻害すること、② これらの作用は対応する金属だけの作用に比しはるかに大きいこと、③ クロロフィリンとトリプシンとの pre-incubation が、その阻害作用を、トリプシンの失活によるものか、部分化学的阻害によるものか、クロロフィリンとトリプシンの複合体が形成されること、④ かかる複合体の形成は酵素の失活を来たすが、それは酸や、硫酸アンモンによって可逆的であること、⑤ 結晶クロロフィリンの全分子量、酵素のイミダザール部にて結合形成されること ⑦ クロロフィリンの作用は拮抗阻害であること、⑧ クロロフィリンの作用はトリプシンの促進剤である CaCl_2 やある濃度の硫酸アンモンによって弱められること ⑨ ある条件下でクロロフィリンの作用はトリプシンの自己溶解を促進するが、これが、トリプシン等によって、トリプシンの自己溶解が促進されること、⑩ クロロフィリン、トリプシンの自己溶解を促進するが、⑪ クロロフィリン、硫酸アンモンの濃度対比が記述すること、作用するよう、明らかになった。

Über die photoperiodische Empfindlichkeit der Kotyledonen von *Pharbitis Nil* Chois.

von Chugo KUJIRAI* und Shun-ichiro IMAMURA**

鯨井忠五*・今村駿一郎**：アサガオ子葉の日長感応性について

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Bei vielen Pflanzen verhalten sich die Jugendformen im physiologischen Geschehen oft ganz anders als die Folgeformen. Dies trifft auch für Photoperiodismus zu, indem die Keimlinge vieler photoperiodisch hervorragend empfindlichen Pflanzen, im Gegensatz zur Folgeform, durch die Abänderung der Tageslänge nicht zur Bildung der Blütenanlagen veranlasst werden können. Schon 1918 hat Klebs darauf hingewiesen, dass jede Pflanze eine bestimmte Periode der vegetativen Entwicklung verlaufen muss, bevor sie den "blühreifen" Zustand erreicht¹⁾. Soweit bisher bekannt wurde, ist *Pharbitis Nil* in dieser Hinsicht die einzige Ausnahme unter Kurztagpflanzen²⁾. In der vorliegenden Arbeit werden einige Beobachtungen mitgeteilt über das photoperiodische Verhalten der Keimlinge dieser Pflanze.

I. Material und Methode

Als Versuchspflanzen dienten *Pharbitis Nil* Chois., Rasse "Violett" und "Tendan", deren photoperiodisches Verhalten bei den Folgeformen eingehend untersucht wurde^{3), 4), 9)}.

Vor dem Aussäen wurden die Samen 30–45 Minuten lang mit konzentrierter Schwefelsäure behandelt und gründlich gewässert, um die Keimung zu beschleunigen und gleichmässig zu machen. Fünzig bis 120 Samen wurden in 4 oder 5 Reihen in hölzernen Kisten von etwa 35 cm. Länge, 20 cm. Breite und 15 cm. Tiefe gesät. Die Mehrzahl keimte gleichzeitig und gleichmässig aus; nur solche Keimlinge wurden zu Versuchen herangezogen. Die allzu früh oder allzu spät auskeimenden Individuen wurden beseitigt. Die Versuche wurden in heizbaren Gewächshäusern teils bei schwankender, teils bei konstanter Temperatur ausgeführt. Die Pflanzen wurden unter kontinuierlicher Beleuchtung gezogen, indem sie in der Nacht mit Glühlampen von 60 Watt belichtet waren. Die Lichtintensität an der Oberfläche der Kotyledonen war etwa 200 Lux.

Die Verdunkelung der Pflanzen wurde derart ausgeführt, dass man die Kulturkisten oder einzelne Pflanzenreihen in diesen mit hölzernen oder metallischen Kisten

* Institut für Chemische Forschung, Kyoto Universität, Kyoto, Japan 京都大学化学研究所

** Laboratorium für Angewandte Botanik, Landwirtschaftliche Fakultät, Kyoto Universität, Kyoto, Japan 京都大学農学部応用植物学研究室

von geeigneter Grösse bedeckt hielt. Auf besondere Durchlüftung wurde dabei nicht geachtet.

Der Versuch wurde im sehr frühen Keimungsstadium ausgeführt, da es sich in der vorliegenden Arbeit um die Induktion der Kotyledonen handelt. Die Kotyledonen, nicht die sich entwickelnde Plumula, kommen dabei als das Organ für Reizaufnahme in Betracht. Um dessen sicher zu sein, wurden in einigen Versuchen die von Kotyledonen befreiten Pflanzen als Kontrolle benutzt, aber die keimblattlosen Keimachsen wuchsen schlecht und gingen oft, besonders im Winter, durch Pilzbefall zugrunde.

Nach der experimentellen Behandlung wurden die Pflanzen unter kontinuierlicher Beleuchtung gezogen und nach etwa 2 Wochen unter Binokularmikroskop untersucht. Die Zahl und die Lage der gebildeten Blütenanlagen an der Hauptachse oder an den durch Beseitigung der Hauptachse zur Entwicklung veranlassten kotyledonaren Achselknospen wurden beobachtet. Bei starker Induktion waren alle Vegetationspunkte zu Blütenanlagen umgewandelt womit die weitere vegetative Entwicklung abgeschlossen war; der Vegetationspunkt der Hauptachse wurde zur Anlegung der endständigen Blütenknospe vollständig verbraucht. Die Zahl der die Blütenanlagen tragenden Pflanzen, die Zahl der Blütenanlagen pro 10 Pflanzen und die Zahl der Pflanzen mit Terminalblüten wurden als Masstabe der Reaktion benutzt. Meistens laufen diese drei Zahlen ungefähr parallel zueinander. In seltenen Fällen von starker Induktion aber tritt eine Unregelmässigkeit ein, indem die Pflanze mit Terminalblüte eine kleinere Zahl der Blütenanlagen führt.

II Versuchsergebnisse

1 Photoperiodische Empfindlichkeit der Kotyledonen in Abhängigkeit mit dem Alter der Keimlinge.

Die Folgepflanze von *Pharbitis Nil* kann durch einmalige, genügend lange Verdunkelung eines einzigen Laubblatts zur Bildung der Blütenanlagen veranlasst werden³⁾. Um die mit dem Alter der Keimlinge vor sich gehende Veränderung der photoperiodischen Reaktion zu prüfen, wurden die Keimlinge an verschiedenen Tagen von ihrem Erscheinen über dem Boden gerechnet, einer Dunkelperiode von 16 stündiger Dauer ausgesetzt und dann unter kontinuierlicher Beleuchtung gezogen. Die Ergebnisse eines Versuchs, der in der Mitte von November ausgeführt wurde, sind in Tabelle 1 wiedergegeben. Am ersten Tage unmittelbar nach der Auskeimung, wenn das Hypokotyl noch eine epinastische Knickung zeigte oder die zwei Kotyledonen noch nicht auseinanderklappten, reagierten die Pflanzen nicht oder sehr schwach gegen Kurztagbehandlung, am zweiten Tage wurden sie tiefer grün und zeigten eine stärkere Reaktion. Mit zunehmendem Alter wurde die Reaktion immer stärker, bis nach 8-12 Tagen ein Maximum erreicht wurde. Mit zunehmendem Alter erschien die erste Blütenanlage an aufeinander folgenden höheren Knoten. In einem anderen Versuch, welcher im Sommer bei konstanter Temperatur von 30° ausgeführt wurde, erreichte die Reaktion schon 4 Tage nach der

Tabelle 1. Photoperiodische Reaktion in Abhängigkeit mit dem Alter der Keimlinge Ausgesät am 12. XI, Versuchsbeginn: 15. XI Eine Dunkelperiode von 16 Stunden.

Rasse	Beobachtete Reaktion	Alter der Keimlinge in Tagen								Licht-kontrolle
		1	2	3	4	6	8	12	12***	
Violett	Blühquotient*	1/10	10/33	10/16	11/18	13/15	18/18	25/25	0/9	0/12
	Blühprozent	10	30	63	61	87	100	100	0	0
	Zahl d. Pflanzen mit Terminalblüte	0	1	1	0	9	17	24	0	0
	Zahl d. Blüten pro zehn Pflanzen	1.0	5.8	16.2	12.2	35.2	49.0	47.2	0	0
	Lage d. ersten Blüte**	—	1.2	1.0	1.1	1.0	1.0	2.1	—	—
Tendan	Blühquotient	0/6	8/20	17/17	16/21	18/18	20/20	9/9	0/7	0/15
	Blühprozent	0	40	100	76	100	100	100	0	0
	Zahl d. Pflanzen mit Terminalblüte	0	0	17	10	14	20	6	0	0
	Zahl d. Blüten pro zehn Pflanzen	0	9.5	45.9	32.8	44.4	61.5	48.9	0	0
	Lage d. ersten Blüten	—	1.0	1.0	1.0	1.1	1.2	3.7	—	—

* Zahl der Pflanzen mit Blütenanlagen/Zahl der Versuchspflanzen

** Durchschnittliche Nummer der Knoten, an denen die erste Blüte zur Anlegung kommt.

*** Kotyledonen beseitigt

Auskeimung ein Maximum, um dann allmählich abzunehmen. Dies mag wohl auf die höhere Temperatur, nämlich auf eine schnellere Entwicklung der Keimlinge und somit auf eine schnellere Alterung der Kotyledonen zurückzuführen sein.

Dass die Induktion durch die Kotyledonen und nicht etwa durch die sich entwickelnden Blättchen der Terminalknospe verursacht wurde, kann man sich leicht auf Grund der Versuchsserie der Tabelle 1 überzeugen, wo durch Beseitigung von Kotyledonen vor dem Versuche nur die sich entwickelnden Plumula dem Dunkeln ausgesetzt war. Die Keimachsen ohne Kotyledonen bildeten niemals Blütenanlage, auch wenn sie 12 Tage nach der Keimung verdunkelt wurden.

2) Photoperiodische Reaktion in Abhängigkeit mit der Dauer der Dunkelperiode.

Die Keimlinge wurden einer Dunkelperiode verschiedener Dauer von 8 bis 16 Stunden mit 2-stündigen Intervallen ausgesetzt. Wie man aus Tabelle 2 ersehen kann, liegt die kritische Dunkelperiode bei der Rasse "Violett" zwischen 8 und 10 Stunden, während sie bei der Rasse "Tendan" kürzer als 8 Stunden ist. Der gefundene Wert der Periode scheint von dem der Folgepflanzen nicht wesentlich abzuweichen^{3),4)}. Es ist bemerkenswert, dass "Tendan" eine in Nord-China einhei-

Tabelle 2 Kritische Dunkelperiode der Kotyledonen. Ausgesät am 19. XII, Versuch: 30. XII, Violett

Rasse	Beobachtete Reaktion	Dauer der Dunkelperiode in Stunden				
		8	10	12	14	16
Violett	Blühquotient	0/11	10/11	12/12	14/14	15/15
	Blühprozent	0	91	100	100	100
	Zahl d. Pflanzen mit Terminalblüte	0	1	5	2	1
	Blütenzahl pro zehn Pflanzen	0	22.7	40.8	30.0	27.3
Tendan	Blühquotient	5/5	8/8	9/9	10/10	9/9
	Blühprozent	100	100	100	100	100
	Zahl d. Pflanzen mit Terminalblüte	0	6	6	8	9
	Blütenzahl pro zehn Pflanzen	18.0	41.2	41.1	46.0	47.8

mische Rasse, einer kürzeren Dunkelperiode d. h. einer längeren Tageslänge angepasst ist⁹⁾.

3 Photoperiodische Reaktion in Abhängigkeit mit der Oberflächengrösse der Kotyledonen.

Der Einfluss der Kotyledonengrösse wurde untersucht, indem man die Kotyledonen durch Abschneiden auf verschiedene Grösse einschränkte. Halbe Kotyledonen erhielt man durch Beseitigung der apikalen Teile und ein viertel Kotyledonen durch Halbierung der basalen Hälften dem Mittelnerv entlang. Die Pflanzen wurden einer oder

Tabelle 3. Photoperiodische Reaktion in Abhängigkeit mit Dimension der Kotyledonen. Ausgesät am 22.V, Versuchsbeginn: 29.V, Violett

Behandlung	16 St. D×1					16 St. D×2*				
Zahl oder Grösse der Kotyledonen	zwei	einer	eine Hälfte	ein Viertel	völlig entfernt	zwei	einer	eine Hälfte	ein Viertel	völlig entfernt
Durchschnittliche Oberfläche der Kotyledonen in cm²	16.2	8.7	5.0	2.6	0	15.9	9.3	4.9	2.6	0
Blühquotient	20/29	10/27	1/29	0/18	0/26	23/23	25/25	18/22	12/22	0/24
Blühprozent	69	37	3	0	0	100	100	82	55	0
Blütenzahl pro zehn Pflanzen	13.2	4.1	1.0	0	0	44.0	35.2	19.1	8.2	0

* Zwei durch eine 8 stündige Lichtperiode getrennte Dunkelperioden von je 16-stündiger Dauer.

zwei Dunkelperioden von 16-stündiger Dauer ausgesetzt. Wie aus Tabelle 3 ersichtlich ist, tritt die Reaktion mit abnehmender Kotyledonengrösse immer schwächer auf, bis ihre vollständige Entfernung den Verlust der photoperiodischen Reaktionsfähigkeit zur Folge hat.

4) Einfluss der Beseitigung der photoperiodisch induzierten Kotyledonen.

Entfernt man das photoperiodisch behandelte Laubblatt von *Pharbitis* oder *Xanthium* zu verschiedenen Zeitpunkten, so nimmt die Blütenbildung mit der Verzögerung der Entblätterung zu^{4),7)}. Um eine ähnliche Möglichkeit an den Kotyledonen zu prüfen, wurde Versuche ausgeführt. Einer von den beiden Kotyledonen wurde mit einem Sack aus schwarzem lichtdichtem Papier eingehüllt, während der andere dem Licht ausgesetzt war. Nach 14 Stunden wurde der Sack entfernt und der verdunkelte Kotyledon wurde sofort oder nach 2, 5 bzw. 10 Stunden beseitigt. In diesem Versuche wurde nur einer und nicht beide Kotyledonen zum experimentellen Eingriff herangezogen, weil die vollständig keimblattlos gemachten Keimachsen oft zugrunde gehen ohne zu wachsen. Wie aus Tabelle 4 ersehen kann, je später der behandelte Kotyledon entfernt wurde, desto mehr Blütenknospen wurden angelegt. Der photoperiodische

Tabelle 4. Einfluss der Beseitigung des induzierten Kotyledons nach 14-stündiger Dunkelperiode auf Blütenbildung. Violett

Datum der Aussaat	Datum der Dunkelbehandlung	Beobachtete Reaktion	Zeit in Stunden nach dem Ende der Dunkelperiode bis zur Beseitigung des induzierten Kotyledons				
			0	2	5	10	∞
22. VI	26. VI	Blühquotient	0/3	0/10	2/8	6/11	21/21
		Blühprozent	0	0	25	55	100
		Zahl d. Pflanzen mit Terminalblüte	0	0	0	0	10
		Blütenzahl pro zehn Pflanzen	0	0	3.8	10.0	41.4
		Blühquotient	0/12	0/7	2/12	7/25	13/13
15. VI	19. VI	Blühprozent	0	0	17	28	100
		Zahl d. Pflanzen mit Terminalblüte	0	0	0	0	3
		Blütenzahl pro zehn Pflanzen	0	0	1.7	3.2	40.7

Reiz, der von dem Kotyledon empfangen wird, scheint innerhalb etwa 19 Stunden zum Vegetationspunkt der Plumula zu gelangen, wie es auch bei der Folgeform der Fall ist⁴⁾.

5) Einfluss der Beleuchtung eines Kotyledons bei Dunkelbehandlung des anderen auf die Induktion.

Bei vielen Kurztagpflanzen erfährt die durch Verdunkelung eines Teils der Pflanze veranlasste Induktion durch die gleichzeitig belichteten Sprosse oder Blätter eine mehr oder weniger starke Hemmung¹⁾. In diesem Versuche erhielt einer der beiden Kotyledonen eine Dunkelbehandlung, während der andere dem Lichte dauernd ausgesetzt blieb. Zum Vergleich standen drei Gruppen der Pflanzen zur Verfügung. In der ersten Gruppe wurde ein Kotyledon beseitigt und der andere mit einem Sack aus schwarzem lichtdichtem Papier 16 Stunden lang bedeckt, während in der zweiten Gruppe der dauernd im Licht gehaltene Kotyledon am Ende der Dunkelperiode beseitigt wurde. Bei den übrigen Pflanzen wurde der belichtete Kotyledon intakt gelassen.

Tabelle 5. Photoperiodische Reaktion, hervorgebracht durch Dunkelbehandlung eines Kotyledons in Abhängigkeit mit dem Vorhandensein des anderen im Licht befindlichen Kotyledon. Dunkelperiod: 16 Stunden. Violett

Datum der Aussaat	Datum des Versuchs	Beobachtete Reaktion	Behandlung des nicht verdunkelten Kotyledons		
			beseitigt vor Verdunkelung des anderen Kotyledons	beseitigt nach Verdunkelung des anderen Kotyledons	nicht beseitigt
15 VI	21. VI	Blühquotient	19/20	12/18	15/22
		Blühprozent	95	67	68
		Zahl d. Pflanzen mit Terminalblüte	0	0	0
		Blütenzahl pro 10 Pflanzen	23.0	11.7	11.8
		Blühquotient	21/25	21/21	14/22
22. VI	26. VI	Blühprozent	84	100	64
		Zahl d. Pflanzen mit Terminalblüte	7	1	2
		Blütenzahl pro 10 Pflanzen	27.6	23.8	11.1

Die Ergebnisse sind in Tabelle 5 zusammengestellt. Beim dauernden Belassen des ununterbrochen dem Lichte ausgesetzten Kotyledons tritt die Reaktion schwächer auf. Die Frage, ob es sich dabei um die Bildung eines Hemmungsstoffes in dem belichteten Kotyledon handelt oder nicht, steht noch offen, da die sich entwickelnde Plumula durch beide Kotyledonen, den behandelten und den nicht behandelten, ernährt wird, wodurch der zugeleitete Reiz selbst eine Schwächung erleiden kann.

6) Photoperiodische Reaktion in Abhängigkeit mit dem Entwicklungszustand der Indikatorknospe.

Die Achselknospen beider Kotyledonen bleiben unentwickelt, solange die Hauptachse ungestört wächst. Sie können aber durch Entfernung der Hauptachse zu Langtrieben

Tabelle 6. Photoperiodische Reaktion in Abhängigkeit mit dem Entwicklungszustand der kotyledonaren Achselknospen. Asugesat am 11. IV; von 21. IV an, die Hauptachse um 4 Uhr nachmittags entfernt; am 24. IV, 16 Stunden lang verdunkelt. Violett

Tag der Beseitigung der Hauptachse	21. IV	22. IV	23. IV	24. IV	25. IV	26. IV	27. IV
Zeitintervall zwischen Dekapitierung und Verdunkelungsbeginn in Tagen	-3*	-2*	-1*	0	1	2	3
Blühquotient	17/19	22/22	19/20	7/17	0/23	0/17	0/23
Blühprozent	90	100	95	41	0	0	0

* Das Minus Zeichen bedeutet dass die Entfernung der Hauptachse der Dunkelbehandlung vorangegangen war.

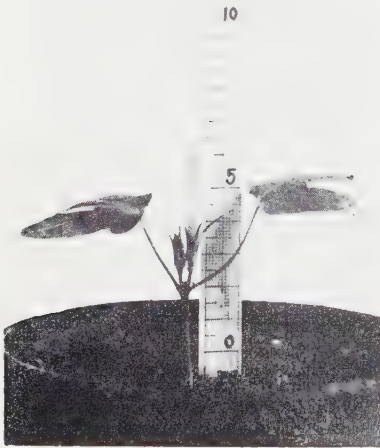


Fig. 1. Kotyledonarblüten von "Tenden" unter Kurztag. Hauptachse entfernt 5 Tage nach der Auskeimung.

werden. Verdunkelt man den Keimling nach Beseitigung der Plumula, so tritt die Blütenanlage an den kotyledonaren Achselknospen auf. Die Reaktion hängt aber stark von dem Entwicklungszustand der Achselknospen, nämlich von dem Zeitpunkt ab, an dem die Plumula entfernt wird. In einem Versuche wurde die Hauptachse zu verschiedener Zeit vor und nach der Dunkelperiode entfernt. Wie aus Tabelle 6 ersichtlich ist, tritt umso stärkere Reaktion ein, je früher die Kotyledonarknospe durch Beseitigung der Hauptachse zur Entwicklung veranlasst wird (vergl. 10). Lässt man die Pflanzen unter Kurztagbedingungen auskeimen, so bilden die Kotyledonarknospen nach Entfernung der Plumula Terminalblüten. In seltenen Fällen werden sie lediglich zu Kotyledonarblüten, ohne Laubblätter auszubilden, wie man aus Fig. 1 ersehen kann.

III Schlussbetrachtung

Die Keimlinge der meisten Pflanzen sind photoperiodisch unempfindlich. Sie lassen sich durch Abänderung der Tageslänge nicht zur Ausbildung der Blütenanlagen veranlassen. Die Keimpflanzen von Sojabohne, Rasse Biloxi, bilden keine Blüten unter den günstigsten Kurztagbedingungen bis zu 4 Wochen nach Auskeimung. Das photoperiodische Verhalten der Keimlinge von *Xanthium saccharatum* wurde neuerdings eingehend untersucht⁶⁾. Die Pflanze ist nicht fähig gegen Kurztag zu reagieren, bevor sie etwa 2 Wochen alt ist, während die Folgepflanze durch eine einzige Dunkelperiode von 12 Stunden zur Bildung der Blütenanlagen gebracht werden kann. Sivori und Went hat aber mitgeteilt, dass der Keimling von *Baeria chrysostoma*, eine Langtagpflanze, photoperiodisch empfindlich ist¹¹⁾.

Im Gegensatz zu den meisten Kurztagpflanzen sind die Keimlinge von *Pharbitis Nil* photoperiodisch sehr empfindlich. Zwar können die eben aus der Erde hervortretenden Keimlinge nicht reagieren, aber am zweiten Tag nach Auskeimen und nachher bilden sie nach einer einzigen Dunkelperiode von genügender Dauer Blütenanlagen. Bei starker Induktion werden alle Vegetationspunkte zu Blütenanlagen umgewandelt und weitere vegetative Entwicklung wird abgeschlossen, indem der Vegetationspunkt der Hauptachse zur Anlegung der endständigen Blütenknospe vollständig verbraucht wird. Die Empfindlichkeit nimmt mit dem Alter zu, bis ein Maximum erreicht wird, um dann wieder allmählich abzunehmen.

Die Frage, warum die meisten Pflanzen im Keimlingsstadium photoperiodisch unempfindlich sind, ist ohne weiteres schwer zu beantworten. In dieser Hinsicht ist die Tatsache von grosser Bedeutung, dass die Keimpflanze von *Pharbitis Nil* unmittelbar nach dem Auskeimen gegen Kurztag nicht reagieren kann. Um zu entscheiden, ob das Ergrünen der Kotyledonen dabei irgendeine Rolle spielt, bedarf eingehenderer Untersuchungen. Jedenfalls mag eine gewisse Veränderung des metabolischen Systems für das Erreichen des "blühreifen" Zustands unentbehrlich sein.

Aus den Resultaten vorliegender Arbeit stellt sich heraus, dass die Keimpflanze von *Pharbitis Nil* in ihrem photoperiodischen Verhalten von den Folgepflanzen nicht wesentlich abweicht. Aber die Möglichkeit kann nicht immer ausgeschlossen sein, dass die beiden Formen sich in Einzelheiten verschieden verhalten.

IV Zusammenfassung

1) Am ersten Tag nach dem Auskeimen ist der Keimling von *Pharbitis Nil* photoperiodisch unempfindlich, aber in folgenden Tagen kann er durch eine einzige Dunkelperiode von genügender Dauer zur Anlegung von Blütenknospen veranlasst werden. Die kritische Dunkelperiode scheint, wie bei der Folgepflanze, zwischen 8 und 9 Stunden zu liegen.

2) Die photoperiodische Reaktion hängt von der Oberfläche der Kotyledonen ab, die verdunkelt wird. Wird diese durch Abschneiden vor der Dunkelperiode reduziert, so ist die Reaktion schwächer.

3) Die Entfernung der behandelten Kotyledonen nach der Dunkelperiode hat eine schwache Reaktion zur Folge. Die Zahl der Blütenanlagen nimmt immer zu, je später der induzierte Kotyledon beseitigt wird.

4) Wenn ein Kotyledon während der Dunkelbehandlung des anderen dem Lichte ausgesetzt wird, wird die Reaktion abgeschwächt.

5) Beseitigt man die Plumula vor der Dunkelperiode, so werden die Blütenanlagen an den sich entwickelnden kotyledonaren Achselknospen gebildet. Bei Beseitigung der Plumula nach der Dunkelbehandlung kommen aber keine oder nur wenige Blütenanlagen zur Ausbildung.

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摘 要

- 1) 発芽当日のアサギサの子葉は暗期に感応したが、翌日には感応性を示し、その日全暗期は成体と同じく 8 ないし 9 時間である。
- 2) 子葉の一部を切りさってその面積を制限すると、面積が小さいほど日長反応は弱くなる。
- 3) 暗期を母として、子葉を除去し、反応を弱くしたが、除去した暗期が長いほど花芽数が増加する。
- 4) 一子葉が暗期を受けている間に他の子葉が光を受けているとき日長反応は弱くなる。
- 5) 主軸を向いた芽生に暗期を母として花芽、子葉の枚数は現れた。暗期を母として主軸を除く子葉枚数は現れた。花芽数は、むしろ減少する。

Oligosaccharides in *Verbascum thapsus* L.

by Shizuo HATTORI* and Shin-ichi HATANAKA*

東京大学理学部植物学教室：ラウドモウズイカの根組織について

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Introduction

Verbascose is an oligosaccharide discovered by Bourquelot and Bridel¹⁾ in 1910 in the root of the mullein (*Verbascum thapsus* L.). It is a tri-galactoside of sucrose, i. e. a non-reducing pentasaccharide²⁾. Recently French³⁾ made a review of frequent co-occurrence of raffinose, stachyose, and verbascose.

In 1954, Hérissé and his co-workers^{4), 5)} published their works on the isolation and structures of higher oligosaccharides of this series. They isolated from the roots of *Verbascum thapsiforme* Shrad stachyose, verbascose, ajugose, heptaose, and octaose, and all the experimental results obtained confirmed the structure of (*O*- α -D-galactopyranosyl-(1 \rightarrow 6))_n-*O*- α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranoside ($n=2$, stachyose; $n=3$, verbascose; etc.), resulting in the correction of the structural formula of verbascose proposed by Murakami²⁾, in which the galactose unit was bounded to C⁴ of the glucose unit.

Almost at the same time and quite independently of the above workers, we were also engaged in the study of oligosaccharides in *Verbascum thapsus* L. We found many oligosaccharides which were considered to belong to raffinose-stachyose type and it was successful to isolate some of them as crystalline form or amorphous powder. Above all, crystals of nonasaccharide was isolated. All these saccharides may be without doubt the same as those found in *V. thapsiforme*.

Afterwards, Courtois and his co-workers^{6), 7), 8)}, and Archambault⁹⁾ published a series of the investigations on the distribution of these oligosaccharides in many plants. Cacao beans¹⁰⁾ contain also oligosaccharides of raffinose series besides those of other series.

The distribution of mono- and oligosaccharides in various stages and organs of *V. thapsus* was also studied, and among the results the occurrence of free galactose, melibiose, and manninotriose is worthy of notice.

Materials and Methods

Authentic specimens

Authentic stachyose was the preparation kindly supplied by Mr. Noguchi who

* Botanical Institute, Faculty of Science, University of Tokyo, Tokyo, Japan. 東京大学理学部植物学教室

had isolated it from the roots of *Lamium album* L. The melibiose and manninotriose were partial hydrolysis products of authentic raffinose and stachyose, respectively.

Paper chromatography

Whatman No. 1 filter paper was used and paper chromatograms were run in a descending procedure and at room temperature (about 10°). As the developing solvents *n*-butanol-pyridine-water (1:1:1.5, V/V) (Solvent A), *n*-butanol-pyridine-water (1 part of pyridine was added to the upper layer of the mixture of 2:1:1.5, V/V (Solvent B) or 3:1:1.5, V/V (Solvent C¹¹), and *n*-butanol-acetic acid-water (4:1:2, V/V) (Solvent D) were selected. Solvent A proved to be very suitable to the separation of higher oligosaccharides. When necessary, multiple ascent technique with one or two solvent systems was carried out with satisfactory results. Reducing sugars were detected by Horrocks' benzidine reagent¹²). For non-reducing, as well as reducing sugars, trichloroacetic acid-benzidine reagent¹³) was adopted. Seliwanoff's reagent¹⁴) was applied for the location of ketose and ketose-containing oligosaccharides.

Extract of sugars

Plant materials, collected in the morning, were chopped into small pieces and extracted a few times with boiling ethanol (80%). The filtrates were combined altogether, concentrated under reduced pressure, and added with 10% lead acetate solution. The precipitate formed was collected by centrifugation and the supernatant was treated with hydrogen sulfide. After the filtrate was aerated for some time, it was concentrated to syrup under reduced pressure. The syrup was submitted to paper as well as column chromatography. In the case of semi-quantitative estimation of sugars, lead acetate treatment was avoided, lest higher oligosaccharides should be precipitated by this deproteinizing agent even a bit.

Results

Occurrence of higher oligosaccharides

Because of small mobilities of higher oligosaccharides on a paper chromatogram their satisfactory separation was achieved only when Solvent A or multiple ascent technique was applied. Examples of paper chromatograms are shown in Fig. 1 and these oligosaccharides were all non-reducing and ketose-containing. On the other hand, it has been observed by French and Wild¹⁵) that the logarithm of the partition function $a = R_f / 1 - R_f$ of a series of homologous oligosaccharides fall on a straight line, when plotted against the polymerization degree. The authors examined this law with the present saccharides and found it held good under various conditions as indicated in Fig. 2.

The sugars which corresponded to Spots 1 and 2 were isolated by large scale paper chromatography followed by total and partial hydrolysis. When they were heated in 2% hydrochloric acid on a boiling water-bath for one hour, hydrolysis was completed and the products proved to be galactose, glucose, and fructose. On milder

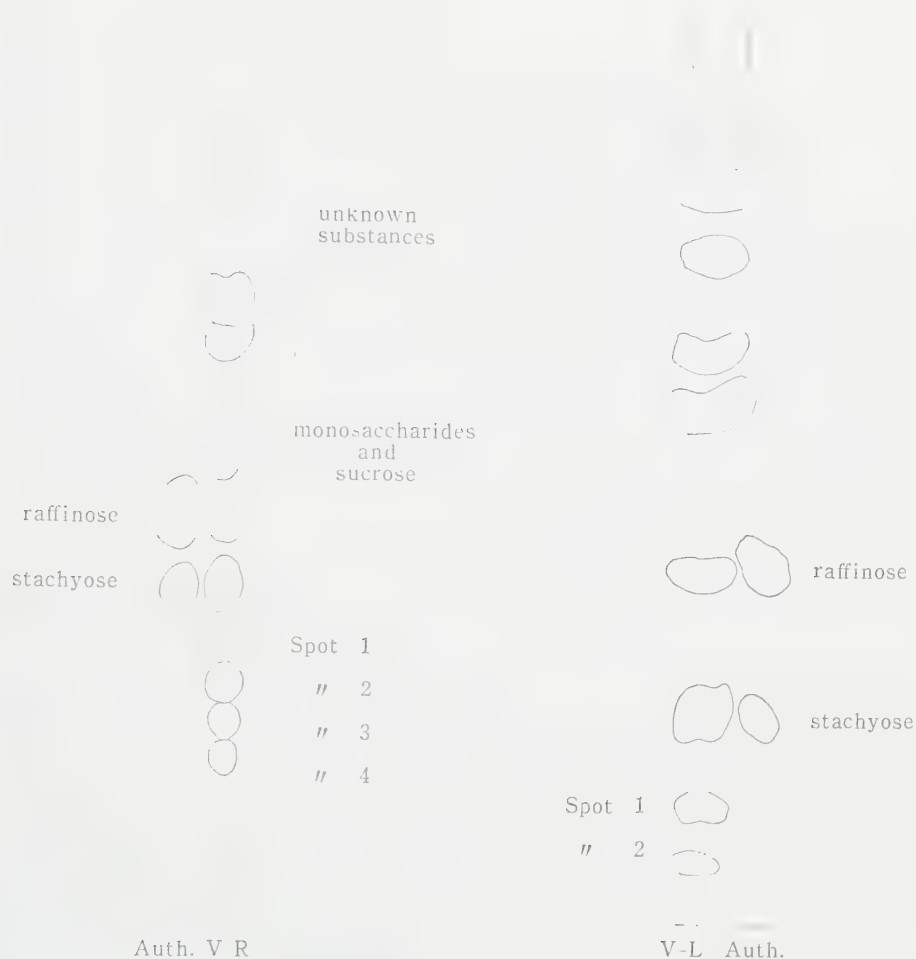


Fig. 1. Paper chromatograms of the extracts of *Verbascum thapsus* L. V-R: root extract, V-L: leaf extract. Auth.: authentic specimens. Solvent: D → A (left), D → B × 2 (right)

condition, i.e. after heating in 2 % hydrochloric acid at 100° for 12 min., many partial hydrolysis products as well as three monosaccharides were detected. These partial hydrolysis products showed no reaction with Seliwanoff's reagent. This result suggested that fructose molecule of these sugars was terminal and acid-labile.

Therefore, it is very likely that all these oligosaccharides belong to raffinose-stachyose type, i.e. Spots 1 and 2 represent verbascose and ajugose, respectively, and Spots 3 and 4, higher saccharides of this family.

Isolation of higher oligosaccharides

For the isolation of oligosaccharides, charcoal-Celite column chromatography based on the method of Whistler and Durso¹⁶⁾ was carried out. The adsorbent used was a mixture of 50 g. active carbon (Wako, "Shirasagi"), heated at 120° for one hour followed by washing with water and drying, and 25 g. Celite (Johns-Manville, New

York). It was kneaded in a beaker with water and packed into a Buchner funnel (30×100 mm.) to make a disk of 25 mm. thick. Prior to the addition of the extract, the column was washed with water. Forty ml. of the syrup derived from 400 g. of fresh root was placed on the column under slightly reduced pressure. To make the displacement of sugars effective, water and 3, 5, 10, 15, 18, and 80 % (V/V) ethanol were applied in succession. The desorption process was followed by paper chromatography. The fractions containing practically only one saccharide were combined and concentrated under reduced pressure to a small volume. After filtration almost

the same quantity of ethanol was added and the whole was left standing in a refrigerator. In a few days, white globular crystals of stachyose, octa- and nonasaccharides precipitated gradually. As the heptasaccharide was not able to be

crystallized by the procedures above, it was further purified paper-chromatographically with Solvent A and treated again as before to give white granules, which did not show any fine crystalline structure under a microscope. Also verbascose and ajugose could not be obtained in crystalline form. The amorphous powder of heptaose melted at 237–9° (in a sealed tube, decomp.) and crystals of octaose, 252° (in a sealed tube, decomp.). Fig. 2 shows a photomicrograph of the crystals of nonaose.**

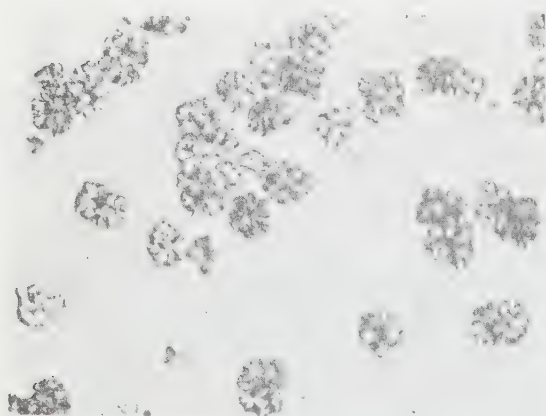


Fig. 3. Crystals of nonasaccharide (×300)

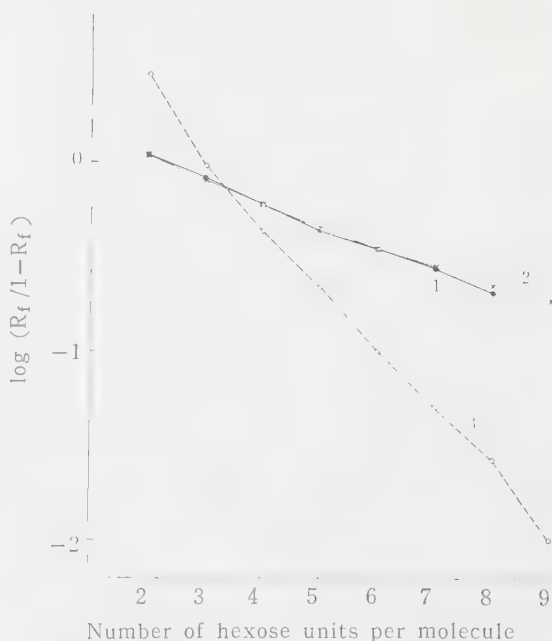


Fig. 2 The relation between R_f -values and chain-length of *Verbascum*-oligosaccharides

1: Solvent A (mean values) 2: Solvent D → A
3: Solvent D → B × 3

** The crystals of nonasaccharide thus obtained proved to be contaminated with a small amount of decaose by paper chromatographical procedure. Hérissé *et al.* recognized also the occurrence of oligosaccharides up to decaose in *V. thapsiforme*.

Table 1. Distribution of sugars in various stages and parts of *Verbascum thapsus* L.

Stage 1: rosette, 2: the plant bearing a short stem, 3: the plant bearing a long stem, 4: the plant bearing buds, 5: the plant bearing unripe fruits
Part R: root, L leaf, La: adult leaf, Ly: young leaf, S: stem, St: stem tip, Sl: lateral stem, B: bud, F: fruit, SD: dried seed

- : <0.01%, + : 0.01-0.05%, ++ : 0.05-0.1%, +++ : 0.1-0.3%, ++++ : 0.3-0.5%, +++++ : 0.5-1.0% (each for fresh weight)

Stage		1		2		3			4			5							
Sugar	Part	R	L	R	La	Ly	S	R	L	S	St	B	R	L	S	St	F	Sl	SD
Fructose		-	++	-	++	++	++	-	+	++	++	++	-	-	++	++	+	+	+
		+	++	+	++	++	+	+	++	++	++	++	+	-	++	++	+	++	++
Glucose and galactose*																			
Sucrose		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Melibiose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	++	+
Raffinose		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	++
Manninotriose		-	+	-	-	++	++	-	-	++	++	++	-	-	++	++	-	++	+
		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Stachyose		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	+
Verbascose		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	-	++	-
Ajucose		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	-	++	-
Heptaose		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	-	++	-
Octaose and higher saccharides		++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	-	++	-

* Glucose and galactose could be separated only in some chromatograms.

Distribution of mono- and oligosaccharides in various parts and stages

Sugars were semi-quantitatively estimated by their spot areas and color densities on paper chromatograms. Higher oligosaccharides were present exclusively in root. Sucrose, raffinose, stachyose, and verbascose were found in every organ, with the exception of fruits and seeds. On the contrary, monosaccharides occurred in leaves and stems much more than in root. Though melibiose was detected in almost every organ, manninotriose was found in stems in which much fructose was present. Melibiose and manninotriose were identified by the comparison of their R_f -values and hydrolysis products with those of authentic specimens. The occurrence of free galactose is interesting. It was observed that there was a discontinuity of the amount between raffinose and higher oligosaccharides. Details of these results are indicated in Table 1.

Data of paper chromatography

As described before, satisfactory separation of higher oligosaccharides was made possible using Solvent A (1:1:1.5, V/V) which was newly prepared by the present authors. Means of the R_f -values were as follows: sucrose, 0.53: raffinose, 0.45 (planteose, 0.43): stachyose, 0.37: verbascose, 0.30: ajugose, 0.26: heptaose, 0.21: octaose, 0.17.

Discussion

As mentioned before, many plants have been reported to contain raffinose and stachyose. It is very interesting, however, to find that so many and higher homologous oligosaccharides are present in a set in the same plant and in the same organ. Some attention should be paid to the discontinuity of the amount of raffinose and higher oligosaccharides, which may suggest that the synthetic mechanism of the former might be different from the latter. Though the biological formation of these oligosaccharides has not yet been completely clarified, it is very likely transgalactosidase plays a most probable role, as does transfructosidase in the case of inulin-type oligosaccharides. If raffinose is formed from sucrose and some galactose donor, stachyose and higher saccharides might successively result by transgalactosidation between thus formed galactose-containing oligosaccharides. On the other hand, melibiose and manninotriose might be regarded as the intermediates in the utilization process of raffinose and stachyose, respectively. Some experimental evidence concerning such transgalactosidation with cell-free extract of *Ajuga nipponensis* Makino has been obtained in our laboratory***. Consequently it must be most important to investigate the enzymatic formation of raffinose. Further study will be carried out in this line.

*** Fujita, Y., unpublished data, presented at the 75th Anniversary Meeting of the Botanical Society of Japan.

封管中, 分解), オクタオース, (6Ga-G-F 結晶, 252°, 封管中, 分解), ノナオース (7Ga-G-F, 結晶)をそれぞれ分離することができた。

またこの植物の種々の生育段階の各部分について糖類の分布を調べた。その結果, 高級少糖類は地上部より地下組織に多く, 単糖類は地上部に多く分布していることが観察した。また茎, 根のガラクトースおよびマンニトリオースが比較的多量に存在することは興味ある事実である。

高級少糖類のペーパークロマトグラフィーの溶媒に, ピリジン-ブタノール-水 (1:1:1.5, V/V)がよい結果を与える。

針葉樹の紅葉および緑葉中のアントシアニン、 ロイコアントシアニンについて

肥 田 美 知 子*

Michiko HIDA*: Studies on Anthocyanidin and Leuco-
anthocyanidin in Autumnal Red Leaves and
Green Leaves of the Conifers.

昭和 33 年 7 月 31 日受付

針葉樹の多くのものには落葉に先だて紅葉現象があらわれる。これは葉内にアントシアニン色素が生じた結果であることは古くから知られていて、^{1) 2) 3) 4) 5) 6) 7) 8) 9) 10) 11) 12) 13) 14) 15) 16) 17) 18) 19) 20) 21) 22) 23) 24) 25) 26) 27) 28) 29) 30) 31) 32) 33) 34) 35) 36) 37) 38) 39) 40) 41) 42) 43) 44) 45) 46) 47) 48) 49) 50) 51) 52) 53) 54) 55) 56) 57) 58) 59) 60) 61) 62) 63) 64) 65) 66) 67) 68) 69) 70) 71) 72) 73) 74) 75) 76) 77) 78) 79) 80) 81) 82) 83) 84) 85) 86) 87) 88) 89) 90) 91) 92) 93) 94) 95) 96) 97) 98) 99) 100)} (林, 阿部, 1955 b, 2),
ラマツ, イススギ, スマスギ, 広葉樹と同様に落葉前に紅葉または黄葉するが、
どではむしろ厳寒時に全樹または一部の枝が紅葉する。このうちスギ、ヒノキの紅葉は主としてカロチノイド色素の分解によるものであると述べられている。

また Bate-Smith ら (1951)⁴⁾ は種々の緑葉中にアントシアニンの前駆物質と考えられているロイコアントシアニン (5, 6) の存在を、
分光光度計によって示した。

それで今回は多くの針葉樹およびイチョウの紅葉・緑葉中のアントシアニン、ロイコアントシアニン、カロチノイドグラフィーと分光光度計によってしらべ、緑葉・紅葉色素と関連し、落葉前・落葉中の色素との関係をしらべた。

材料および方法

材料は京都大学農学部演習林、京都大学理学部植物園、
1. 紅葉の色素

色素の抽出は広葉樹の紅葉に用いられたと同様
した紅葉を細切し、これに重量の約 10 倍量の 1
%メタノール塩酸を加え、一昼夜浸し色素を抽出
する。この抽出液を試料として、幅 1 cm., 長さ
15 cm. の東洋濾紙 No. 50 の原点につけ乾燥後、
水酢酸:36%塩酸:水 (5:1:5), 水酢酸:36%塩
酸:水 (3:1:8) およびアセトン:5%塩酸 (1:1)
で展開し、*R_F* 値を測定した。次に抽出液に同量の
20%塩酸を加え 95° の温浴上で半時間加熱する。
その後水酢酸を加え、
ソアミルアルコールを加えよく振とうする。この
の配糖体は分解してアグリコンと糖になり、前者
を試料とし、前同様にして展開し *R_F* 値を測定した。
其後それぞれの *R_F* 値を持つ分離色素の部分を取り
とり分光光度計にかけたが、^{10), 11)} それには切り
とった色素部分の濾紙をガラス板にはさみ、セル
ホルダーに入れ、波長 500 mμ から 600 mμ の間の各波長
での吸収を測定した。

* 大阪女子大学生物学教室 Biological Laboratory of Osaka Women's University.

Table I. Kinds of anthocyanidin separated from the coloured and the green leaves of the conifers and Ginkgoaceae

Families	Species	Kinds of Anthocyanidin separated from	
		Green Leaves	Coloured Leaves
Ginkgoaceae	<i>Ginkgo biloba</i>	D	—
Taxaceae	<i>Taxus cuspidata</i>	C	C
	<i>T. cuspidata</i> var. <i>nana</i>	C	
	<i>T. floridana</i>	C, D	
	<i>Torreya nucifera</i>		C, D _s
Podocarpaceae	<i>Podocarpus chinensis</i>	C, D _s	.
	<i>P. Nagi</i>	C	.
Araucariaceae	<i>Agathis</i> sp.		C
	<i>Araucaria Braciliana</i>	C, D	C _s , D
Cephalotaxaceae	<i>Cephalotaxus drupacea</i>	C, D	C, D
	<i>C. nana</i>	C, D	
Pinaceae	<i>Abies firma</i>	D	
	<i>A. homolepis</i>	D	
	<i>Cedrus Deodara</i>	D	.
	<i>Keteleeria Davidiana</i>	C, D _s	
	<i>Larix leptolepis</i>	D	
	<i>Picea excelsa</i>	C, D	.
	<i>P. Koyamai</i>	C	.
	<i>Pinus amamiana</i>	D	.
	<i>P. densiflora</i>	D	.
	<i>P. pentaphylla</i>	D	.
	<i>P. rigida</i>	D	.
	<i>P. Thunbergii</i>	C, D _s	.
	<i>Pseudolarix Kaempferi</i>	(C, D _s)	C, D _s
	<i>Pseudotsuga japonica</i>	D	.
	<i>Tsuga Sieboldii</i>	D	.
Taxodiaceae	<i>Cryptomeria japonica</i>	C	C
	<i>C. japonica</i> f. <i>alboepicata</i>	C	
	<i>Cunninghamia Conishii</i>	C	
	<i>C. lanceolata</i>	C	C
	<i>Glyptostrobus</i> sp.	C, D	C _s , D
	<i>Metasequoia glyptostroboides</i>	C, D	C, D _s
	<i>Sciadopitys verticillata</i>	C _s , D	C _s , D
	<i>Sequoia sempervirens</i>	C, D _s	C, D _s
	<i>Sequoiadendron giganteum</i>	C, D _s	C, D _s
	<i>Taiwania cryptomerioides</i>	C _w	C _w
	<i>Taxodium distichum</i>	C, D	C, D
Cupressaceae	<i>Biota orientalis</i>	D _w	C, D _s
	<i>Chamaecyparis formosana</i>	C, D	C, D
	<i>C. obtusa</i>	C	C
	<i>C. obtusa</i> var. <i>breviramea</i>		C
	<i>C. pisifera</i>	C	C
	<i>C. pisifera</i> var. <i>squarrosa</i>	C	C
	<i>Juniperus chinensis</i>	C, D _s	.
	<i>J. rigida</i>	C	.
	<i>Thuja occidentalis</i>	C, D _s	C, D _s
	<i>Thujaopsis dolabrata</i>	C	C
	<i>Cryptomeria/Metasequoia</i>		C
	<i>Sequoia/Metasequoia</i>		C, D _s
	<i>Sequoiadendron/Metasequoia</i>		C, D _s

C: Cyanidin, D: Delphinidin, s: strong, w: weak, —: no Anthocyanidin ×: no Coloured Leaves, (): trace, []: separated from 1% methanolic HCl extract. *Cryptomeria/Metasequoia*: *Cryptomeria* is the scion, and *Metasequoia* the stock.

Summary

Anthocyanidins in the coloured leaves and leuco-anthocyanidins in the green leaves of the conifers were investigated by means of paper chromatography and spectrophotometry.

The results can be summarized as follows :

1. The red colours of the conifers' autumnal leaves seem to be mostly due to anthocyanins.

2. The anthocyanidin present in the coloured leaves is identified with cyanidin or delphinidin; some species contain both cyanidin and delphinidin, some, cyanidin, but none contains delphinidin. These anthocyanidins may not be produced solely from the anthocyanins but from the leuco-anthocyanins co-occurring in the autumnal leaves.

3. The leuco-anthocyanin present in conifers' green leaves is converted into cyanidin or delphinidin by heating with 2N-HCl.

4. Anthocyanidins formed from leuco-anthocyanins in the green leaves are generally the same as found in the coloured leaves of the same species.

5. The kind of anthocyanidin and leuco-anthocyanidin found in the coloured and the green leaves is characteristic of systematic position of the plants; there are more species which contain leuco-delphinidin than those which produce both delphinidin and cyanidin among Pinaceae, while species which form both delphinidin and cyanidin are more common than those giving cyanidin alone among Taxodiaceae and Cupressaceae.

6. Leaves of major species of Pinaceae give delphinidin alone when heated with HCl and do not change their colour in autumn or in winter.

7. The systematic distribution of anthocyanidin and leuco-anthocyanidin in the coloured and the green leaves suggested that *Metasequoia* is closely related to *Sequoia* and *Sequoiadendron* among Taxodiaceae.

Short Communication

Increase in Percentage of Gibberellin-induced Dark Germination of Tobacco Seeds by N-compounds.

by Tohru HASHIMOTO*

橋本 徹*: ジベレリンによっておこるタバコ種子の暗発芽におよぼす窒素化合物の影響

Received November 11, 1958

It was reported by Ogawara and Ono that gibberellin induced the germination of tobacco seeds without exposure to light²⁾. We have found that this effect of gibberellin is largely intensified by N-compounds.

Seeds of *Nicotiana Tabacum* L., "Bright Yellow", obtained at Tateyama (Chiba) were studied. These ordinarily require light exposure for germination. Effects of potassium nitrate and ammonium nitrate on the germination of the seeds were observed in the presence or absence of gibberellin. Tested concentrations of gibberellin (gibberellic acid and gibberellin A mixture) and N-compounds are 30 p.p.m. and 0.02 M, respectively. Two layers of filter paper were placed in each petri dish and soaked with the solutions mentioned in Table 1. About 150 seeds were sown in each of these petri dishes, were kept at 25° in total darkness for seven days to allow seeds to germinate, and percentages of germination were determined.

Table 1. Percentage of germination of tobacco seeds.

Solution	water	Gib.	KNO ₃	Gib.+KNO ₃	NH ₄ NO ₃	Gib.+NH ₄ NO ₃
lot 1	0	8.5	3.2	55	4.5	53
lot 2	0	13	1.6	56	7.6	43

As shown in Table 1, gibberellin induces the germination of tobacco seeds without light exposure, and this effect of gibberellin is intensified to a large extent by the addition of potassium nitrate and ammonium nitrate.

Ogawara and Ono reported previously that the effect of light on tobacco seed germination was larger in the presence than in the absence of N-compounds including potassium nitrate and ammonium nitrate¹⁾. According to the present study these N-compounds increase the rate of gibberellin-induced seed germination, too. This suggests that N-compounds accelerate the physiological steps which can be influenced

* Biological Institute, College of General Education, University of Tokyo, Meguro, Tokyo, Japan. 東京大学教養学部生物学教室

Table 2. Percentage of germination of tobacco seeds.

N Comp. compound concn. (Mol)		Gib. concn. (p.p.m.)					
		0	0.3	1.0	3	10	30
KNO ₃	0	1.7	3.6	2.8	10.1	9.9	40.2
	0.04	1.6	2.5	3.0	5.4	20.0	66.5
	0.08	4.1	4.9	4.9	7.8	30.9	68.8
NH ₄ NO ₃	0	2.9	3.3	1.9	4.3	12.2	58.1
	0.04	3.0	7.1	3.7	43.3	77.0	79.6
	0.08	4.9	36.3	38.3	70.6	77.9	78.7

by light or gibberellin.

Table 2, obtained by another series of experiments, reveals that by treating tobacco seeds with gibberellin in the presence of N-compounds, the effect of even very small amounts of gibberellin can be detected.

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———, Japanese Gibberellin Research Association, First Symposium on Gibberellin Research
in Japan (1957).

雑 録

改良型ポトメーターについて

A Modified Potometer for a Dual Purpose*

by Arcot VISWANATHAN**

植物の蒸散で水は重力に逆らつて吸いあげられる。わたしは、もぐり人形が密閉した容器内で圧力の変化により浮き沈みする原理を利用して、蒸散の有様をより印象的に示す装置を考案した。

もぐり人形は、水に沈むと浮くのを繰り返す。高さ 25 cm. に入れた時、底まで沈むのに少なくとも 5 秒はかかるように比重を調節する。厚さ 2.5 cm. のゴム栓に孔 (直径 3~4 mm.) をいくつかあけ、それでピンに蓋をし、孔も水でいっぱいになるようにする。ゴム栓の右端の孔に、コック付きの肉厚の毛細管 A (内径 1.5 mm.; 長さ 22 cm.) を、端から 6 cm. のところで直角に曲げてさしこみ、左端の孔には長さ数 cm. のガラス管 B を、ゴム栓の中心から A より 2 cm. だけさしこむ。

実験材料は何でもよいが、わたしは、長さ 30 cm. のホウセンカを 5 本 (葉は約 50 枚) 用いた。通気管は、試験が長く水が蒸発しすぎないように葉を一時間水につけ水中で切つて調整の孔をさしこみ、上向きに調整し、水で満たし密閉する。葉の葉脈をさしこむ孔のすぐ上につめ、まわりをロウで厚くおおう。口を全部閉じた時、浮きあがらせたもぐり人形が 5 分以内に沈むかわりに直ちに出口が、一考してよい。用いるロウは植物に害がなく、ほどほどに柔らかいなら何でもよいが、わたしは蜜ロウにヒマシ油を適当に加えて混合して一週間放置したものを用いた。

実験 1、従来のポトメーターによる実験であるが、コックを開き、B からピペットで水を注入し A の空気をおいだす。A の端を指で押さえ、B にも水を満たして密封し、ガラス栓を挿入する。す

ると植物の蒸散のために A 内の水はコックの方に向つてこみだす。

実験 2、今度は A にゴム管 (長さ 25 cm.) をつけ、図のように上方に曲げておき、これにいくらか水がたまるまで B から水を注ぐ。B も水でいっぱいにして、コックを開き、水は A の端からこみだす。このとき、植物の蒸散で A 内の水はコックの方に向つてこみだす。



もぐり人形は、水に沈むと浮くのを繰り返す。比重を適当に調節するなら、人形が浮上している時コックを少し開いて 10 秒間放置し、沈み始めるとすぐコックを閉じればゆっくりと沈んでゆき、14 秒以内に底に着くようになる。

さて、もぐり人形を底におき、出口を全部閉じておくと蒸散のためにビン内の水が奪われ、圧力がどんどん減っていくので、わずかに数分後にもぐ

* 本文は原著者の依頼により紹介した。

** The New College, Madras 14, India.

り人形が浮きあがる。実験を短時間に終わらせるにはもぐり人形を圧力の変化に対しなるべく反応しやすいうようにしてやる必要がある。以下、この人形を製作するにあたり、必要となる材料と製作方法を述べる。

この実験を成功させる秘訣は、もぐり人形内の気泡は別として、装置から完全に気泡を除くこと

である。直射日光があたると、人形内の空気が膨脹して浮きあがることもあるから直射光をさけて「白くし晴天下に、きこ散光下行」(『行状』上巻)と、微風が速く、先故・肥がすくしく初日のように、厚地俄に出て来ようとする。

(戸塚 績 訳)

国際海洋学会議 (International Oceanographic Congress) の開催について

アメリカ科学振興会は UNESCO 及び ICSU の SOCR (Scientific Organization for the Conservation of Resources) の一環として、12月まで、New York の国連ビルにおいて国際海洋学会議を開催する。

海洋学・海洋生物学に関する基礎科学全分野にわたる諸問題が論ぜられ、下記の2部門を含む5部門の Symposium が開かれる。各部門2日ずつの日程で、毎日午前は3人の指定構演者の講演、午後は提出論文についての発表討論が行なわれる(5, 6日、休会)

4. Cycles of organic and inorganic substances in the sea. (9 || 10, 11 || 1)
5. The marine life regime. The popu-

lation of the sea. (9月 2, 3日)

同一論文を提出した人は次のような規定で原稿を作製し、提出することができる。

- (1) 送り先: Dr. Mary Sears, Woods Hole
Oceanographic Institution, Woods Hole,
Massachusetts, U. S. A.
- (2) 締切期日 (必着日): 1959 年 1 月 31 日まで
に abstract (200 語以内, 英・仏・独・露ま
たはスペイン語)。12×9 インチのタイプ用紙に
上・下 1 インチずつ, 左 1.5 インチ, 右 0.5 イ
ンチあけて, double space で type-write
する。

(東大農学部 水産植物学教室 新崎盛敏)

本 会 記 事

第 23 回 大 会

10 月 25 日(土)—27 日(月)の 3 日間、第 23 回大会は九州大学において開催された。これにききだち 24 日(金)の夜には、評議員会が開かれ、別項のような内容が報告、承認された。翌 25 日は午前 9 時半から A, B, C, D の 4 会場にわかれて一般講演が開催された。これらの 4 会場は新築されたばかりの防音教室で、近くの板付飛行場から飛来するジェット機騒音も、遠くから飛来することなく入るのを防止していた。26 日からは午前中、シンポジウムが 3 つの部内で行われ、午後からは一般講演が前日に引きつづき開かれた。この日は市内に降った大雪のおかげで、南国の地—福岡も異常な寒さで参加者一同をふるえめからせた。最終日の 27 日も前日と同じく、午前中にシンポジウム、午後には一般講演がおこなわれた。午後 3 時すべての一般講演が終ると、記念撮影につづいて総会が工学部の大講堂で開かれた。総会後、午後 6 時より福岡帝國ホテルにおいて、来日中のインド・ラクノーのビルパール・サーニ化石植物学研究所の名譽所長であるサーニ夫人を幹事として、同所、高野、高野、高野(幹)で懇親会が開催された。こうして正会員、臨時会員 500 名が参加、194 の一般講演と、24 のシンポジウムの話題が提供された第 23 回福岡大会は盛会のうちに終了した。翌 28 日、希望者は 3 班に分かれ、A 班は市内見学、B 班は阿蘇・別府、C 班は雲仙・長崎の見学旅行に出発した。なお今回の大会に始めて被同作者の市内見学が 27 日におこなわれ、学会の歴史の上に一つのエポックを作った。

福岡大会は九州支部の方かたの努力のおかげで、まことにスムーズにそしてスマートに運営され、委員会員に多大の協力を与え、今後の大会運営のモデルという印象を受けた。

評議員会 (10 月 24 日午後 4 時半、福岡日活ホテル)

出席者：評議員 18 名(欠席 7 名)、会長、幹事長、幹事 3 名、大会準備委員 2 名。

会長の挨拶につづいて、幹事長から次の諸事項

についての報告がおこなわれ、種々討論ののちに承認された。

1. 役員移動。2. 評議員改選。3. 現在会員の状況。4. 32 年度会計。5. 33 年度会計中間報告。6. 34 年度予算案。7. 植物学雑誌刊行経過および予定。8. 図書の交換・寄贈の状況。9. 各種奨励金候補の推薦状況。

さらに東北支部の神保忠男評議員から、次回の大会は仙台で 9 月 4, 5, 6 日の 3 日間、東北大学教養学部で開く予定であるという報告がなされた。なお 35 年度の大会は一応近畿支部で開くことになった。

ついで次期会長候補の選挙に入り、服部静夫、木村重雄、高野高野(幹)氏が立候補した。更に、部省学術奨励審議会科学研究費等分科審議会委員候補として、三輪知夫、前川文夫、原寛の三氏を選んだ。

総 会 (10 月 27 日(月)午後 4 時、九州大学工学部大講堂)。

会長の挨拶につづいて、幹事長から次の諸事項についての報告がなされた。

- (1) 役員移動の報告および承認(評議員、幹事長、幹事、編集委員：本年 4 月号掲載)
- (2) 現在会員の状況報告(昭和 33 年 9 月 30 日現在：1173 名。うち名譽会員 21 名、特別会員 22 名。外国通信会員 4 名、終身会員 51 名、通常会員 1075 名)。
- (3) 会員移動の報告(昭和 32 年 10 月 1 日～昭和 33 年 9 月 30 日、新入会 109 名、死亡 5 名、退会 19 名、除名 63 名、差引増加 22 名)。
- (4) 植物学雑誌刊行経過および予定の報告。

(附表)

- (5) 図書の交換・寄贈の状況報告(交換：国外受理 83, 国外発送 76, 国内受理 37, 国内発送 32, 寄贈：国外受理 8, 国外発送 2, 国内受理 43, 国内発送 4, その他発送 6, 予約購読 331)。
- (6) 昭和 32 年度決算の報告(昭和 32 年 1 月～昭和 32 年 12 月、本年 2 月号掲載)。
- (7) 来年度大会に関する報告(前記評議員会の項参照)。

	論文数	ページ数
1955 年度	64	372
1956 年度	97	602
1957 年度	77	438
1958 年度 1 月号	7	36
2 月号	7	42
3 月号	6	48
4 月号	6	38
5 月号	5	36
6 月号	5	34
7-8 月号	8	54
9 月号	4	30
10 月号 (予定)	5	40
11-12 月号 (予定)	10	90
	63	448

一 般 講 演

分類・形態・細胞・遺伝・地理

- 倉林正尚：オオバナノエンレイソウにおける種内分化の機構の要因
- とオオタチカモジグサの自然雑種
- 上井田幸郎：タデ属植物の発生学的研究 I
- 澤田義康：ユリ科植物の花粉発芽とアミノ酸について
- 鳥山英雄：オジギソウの細胞生理学的研究 (第 10 報)
- 馬場三吾：ジャガイモのカルス形成の際における組織呼吸の阻害と細胞分裂について
- 山崎照俊：酵母菌の出芽と X 線感受性について
- 佐々木正人：シャジクモ類の細胞学的研究 VII オオシャジクモ (*Chara corallina*) の造精器の成熟度と分裂ひん度との関係および分裂ひん度の日中変化
- 長松四郎：スミレモ科植物の細胞学的観察
- 神谷 平：藻類ペン毛の mastigonemes の電顕像
- 宮井あい子：植物細胞のオスミューム固定について

- ての電子顕微鏡的研究
- 植物の組織化学的研究
- 山岸秀夫：凍結真空乾燥法による *Spirogyra ellipsospora* の固定
- 横村英一： *Vicia faba* の根端細胞核中の DNA 量
- による desoxyribo 核酸の微量定量
- 山崎典子：こあつもり染色体の DNase 処理
- 植田勝巳：数種緑藻の pyrenoid の構造について
- 植田利喜造・村上悟：葉緑体の発達に関する電子顕微鏡的研究
- Cyanophyta 細胞の形成
- 寺田 保：フラスモの電子顕微鏡的研究
- 寺田 保：電子顕微鏡による変形菌の微細構造に関する研究 (III)
- 上野実朗：電子顕微鏡による針葉樹花粉膜の微細構造
- 粉川昭平：圧縮変形されたミツガシワ遺体の復元
- 三木 茂：遺体から見た邦産裸子植物
- 川崎次男：ヘラシダ属 *Diplazium* の有性世代と二、三の分類学的性質について
- 北村四郎：アフガニスタン野生植物 (カラコルム・ヒマラヤ)
- 北村四郎：欧亚大陸の東西栽培植物の交流 (カラコルム・ヒマラヤ)
- 足川大録：Jungermanniaceae 植物の油体
- 新 敏夫：琉球列島のセン類 III. Mniaceae (チョウチンゴケ科) の生態・分布
- 越智春美：日本産カサゴケ科セン類にみられる分布型について II. カサゴケ亜科 (Bryoideae) について
- 鈴木兵二：本邦ハリミズゴケ類の分類と分布
- 砂山真理子： *Saprolegnia parasitica* について
- 曾根田正己： *Prototheca* 属に関する研究
- 椿 啓介： *Oedoecephalum* 属の子嚢世代について
- 宮井あい子：モエシマンシ初期前葉体、および細胞

の分裂と伸長との関係

伊藤道夫：モエジマシダ前葉体の細胞分化と造精器形成との関係

中沢信午：シダ原系体における“頂化”と“基化”

三宅昭吉：ワケナシ・ワケナシ科植物の系統について

西岡泰三：タンポポ科の核型分析 (4 報)

辰野誠次：Tukakia lepidiozoides の染色体とセントアイ類染色体進化の一考察

土屋 工：オオムギに見出された long chromosomes

村上 覚：九州地方産 Kalimeris incisa 群の染色体数と地理的分布について

酒井文三：アゼトウナ雑種集団の核型の変異

佐保 貴：エンレイソウ (Trillium smallii Maxim.) の 1 集団における染色体組成の分析

福島博・丸山晃：Chroococcus (ラン藻) の分類

福島博・小林艶子：Cymbella sumatrensis Hustedt (ケイ藻) について

加崎英男：日本産シャジクモ類 第 12 報 (再びホソバシャジクモについて)

吉田道雄：九州産緑色ワケナシ科植物の系統について

瀬田良三・広瀬弘幸：日本産カリモズク Batrachospermum moniliforme Roth の生活史について

千原光雄：カギノリ科植物の生活環—とくにタマキタケとシロハケマイノミヤナギを中心として—

野田義典：日本産の海藻 I. アザミノ藻類

広瀬弘幸：藻類にみられる遊走細胞の放出孔の構造とその類縁関係の意味

山藤千春：四国産トウモロコシ科植物の系統について

竹中 要：オーストラリア種 Nicotiana gossei とアメリカ種数種との雑種

広本一由：シメジに関する研究

伊藤太郎：アカパンカビ子実体形成時における雌雄型による NO_3^- および NH_4^+ の消費について

増岡弘吉：秋まき小麦にみられた分枝性異常穂の出現について

斎藤真太郎：セン類のさく歯の発生学的研究 ヨツバゴケについて

加崎英男・岩崎尚彦：Nitella inokasiraensis の生長点の分化と器管形成

井上隆吉：アカザ科の一次管束系について

島袋敬一：イネの根の組織の発達

原 襄：外巻葉の形成とツツジ科の系統

伊藤 洋：らせん葉序の成立についての一つの考察

熊沢正夫：双子葉類前葉配列変化に関する続報

生理・生化・生態

佐田正三・大 島 正：トウモロコシの根の生理

佐田正三・大 島 正：トウモロコシの根の生理について

林 克己：トウモロコシの芽ばえにおいてアンモニアの同化におよぼす K⁺ イオン効果

岡本 尚：ミトリササゲ子葉から芽ばえへの陽イオンの輸送

小嶋辰男：サツマイモのりん酸の吸収と必須カチオン欠乏との関係

井上泰栄・田中忠治：うるち、もちおよび糖質トウモロコシの胚乳の pH、無機りんなどの消長と生育・生育環境との関係、生育環境と生育化合物

前田敏・小島均：葉面吸収後の物質の移行経路について

前田敏・小島均：根の生理による放射性りん酸の吸収について

前田 稔：植物葉片の凍結曲線に現われる耐寒型と非耐寒型

田中 正・小島 均：Comidia を形成する放線菌 No. 602 株について

信夫隆治：放線菌の分類に用いられる生理学的特徴の再検討

山田 保：葉粒菌の suspension のナタネ幼植物の発育におよぼす影響

山根銀五郎・東 四郎：根粒菌 Rhizobium のマメ科植物の根粒形成におよぼす作用

石川敏・寺岡宏・宇佐美正一郎：春まきコムギ発芽期の呼吸系について

桑山勇寿男・宇佐美正一郎：水稻発芽期の末端呼吸酵素について

仲尾澄子・宇佐美正一郎・鈴木屋：細菌のフェーザル酸化系について Azotobacter によるヒドラジンの酸化

吉家やす子・森健志：Acetobacter suboxydans の末端呼吸系について

岩崎勇一・森健志：脱酸素反応におけるチトクロームの関与について

平井一男・森健志：耐塩菌のチトクロームの変換現象について (II)

森健志・岩崎寿：Thiobacillus thiooxidans のいおう酸化

山田三郎・山田三郎：リボ核酸の意義

須田省三：微生物の生長におよぼすインドール酢酸の作用

山田三郎・山田三郎：リボ核酸の意義

作用におよぼす鉄イオンの影響

和田俊司・長尾昌之：イネ子葉鞘のインドール酢酸酸化酵素

勝見允行：1, 4-デヒドロナフトエ酸-(1) の生理作用

加藤次郎・三井哲夫： α -ナフトエ酸のハロゲンならびにニトロ基置換体の伸長およびカルス形成作用

山田三郎・山田三郎：リボ核酸の意義

村上 浩：ジベレリンによる根の伸長促進作用

山田三郎・山田三郎：リボ核酸の意義

おぼすジベレリンの効果

小西通夫・崎山登世子：えん麦子葉鞘切片の伸長作用

今村駿一郎・小川幸持・奥田光郎：アサガオわい

石川重夫：種子発芽におよぼすクマリン類縁物質の作用

山田三郎・山田三郎：リボ核酸の意義

る作用物質の研究 I. ツクリタケ (*Agaricus bisporus* (Lange) Sing.) の子実体生長促進物質

山田三郎・山田三郎：リボ核酸の意義

小西通夫・萩本宏：担子菌子実体の生長を促進する作用物質の研究 II. ツクリタケ (*Agaricus bisporus* (Lange) Sing.) の子実体に存在するオーキシシンについて

今井百里江子：放線菌のこんばく培地上の発育

山本日本・谷野淳一：*Phytophthora infestans* (Mont.) DaBary 菌の胞子形成について

大槻虎男：好ちょう糸状菌の研究 金属鉄研磨面における発育について

尾辻 望：大腸菌の lysed-protoplast による酵素形成と核酸塩基 analogues の作用

堀田康雄：イースト細胞におけるたんぱく質の代謝的安定性とたんぱく合成との関連

桃谷好英：種子たんぱく質における種属特異性

山田三郎・山田三郎：リボ核酸の意義

じかびのミトコンドリアの酸化に伴なう脂肪酸エステル化反応

秦野節司：変形体の ATP 含量と 2, 3 の生理的条件下におけるその消長について (III)

山田三郎・山田三郎：リボ核酸の意義

転移反応 (5) 光化学的還元物質の生成との関連

服部明彦・藤田善彦：ラン藻および紅藻の phyco-bilin pigments について

代谷次夫：タバコの葉におけるホリフェノールオキシダーゼの作用

山田三郎・山田三郎：リボ核酸の意義

る生化学的研究 (I)

代谷 康：高等植物の黒変現象 ダフネチンの関与する黒変現象

山田三郎・山田三郎：リボ核酸の意義

服部静夫・駒嶋稔：ハッシュウマメに存在する新アミノ酸

滝本敦・池田勝彦：アサガオ子葉の日長感応に対する弱光の影響

奥田光郎：種間および属間のつぎ木による開花刺激作用

小林万寿男：茎の極性発現、とくに発根に関する研究

山田三郎・山田三郎：リボ核酸の意義

買來章輔：花芽分化期および開花期における土壌水分条件の変化に伴なうゲイズの水分生理

堀江格郎：ムラサキツユクサの開花における花卉の行動

飛田博温・中山至大・奥村重雄：花芽形成の光可逆性に対する kinetin の効果

中山至大：花芽形成の光可逆性

藤山良平：光刺激による胚軸の生長抑制とそれによる発根の促進

江刺洋司・長尾昌之：シュウカイドウの無性芽形成 (第 4 報) 日長処理に伴なう生長促進および抑制物質の消長

小田健二：ジャシクモの膜電位を構成する 2 種のイオンチャンネル

山田三郎・山田三郎：リボ核酸の意義

導速度

柴岡孝雄：シャジクモ節部の伝達の機作

林俊郎・土坪英治：シャジクモ類の原形質分離と
原形質流動

岸本卯一郎：Chara の節間細胞の電位特性と原形
質流動

阿部重美：原形質流動と SH

香山時彦：異常環境下における霊菌の行動

尾形英二：海藻の呼吸と塩分濃度との関係

高田忠吉：西日本に分布する水生植物の生長と水深傾
斜環境における発育について

貝原友次郎：酵母細胞膜の粘質層について

西上義一：酵母に対する 2,4-D の影響

瀬野保二・芦田譲治：酵母の銅抵抗性の遺伝

荒勝 豊：酵母の銅抵抗性を規定する要因につい
て

村山徹郎・芦田譲治：銅耐性酵母の有機酸および
アミノ酸代謝

岩田忠吉・内田信夫・芦田譲治：銅耐性酵母の硫酸
酸および亜硫酸還元系について

内田信夫・岩田忠吉・芦田譲治： $S_2O_4^{2-}$ による銅
耐性酵母のいおう代謝の研究

中村運・芦田譲治：酵母のカドミウム耐性

柳田直彦：酵母の W 因子の誘導、とくにリボ核酸
との関係

徳野真一・坂本敏彦・高田英夫：酵母食塩抵抗菌
における原形質膜リボ核酸と色素・イオンの
透入 I

高田英夫：酵母食塩抵抗菌における原形質膜リ
ボ核酸と色素・イオンの透入 II

永田 進：マンガン化合物による呼吸阻害酵母菌
の誘導について

宮本義男：微生物によるろう・パラフィンの分解
(附) 2, 3 のポリマーの炭素源としての意味

志平依久予：緑藻の swarmer の運動と光

中谷 茂：ラン藻の純粋分離法について

渡辺篤・服部明彦・藤田善彦・清原千里：温泉利
用によるラン藻の生育的増殖について

西崎友一・高田忠吉・高田英夫(第 2 報) 光の吸収
と反応経過

杉 野守・山下孝介：葉緑素を欠く一粒系コムギ
の葉変異体に対する低濃度処理による葉緑素形
成(予報)

藤 茂宏・鈴木洋太郎・佐藤公行：Euglena の葉
緑体反応

岩田忠吉・西山武：種子および発芽時の花の浸透
調節の成立

高山伊佐男・河野清：吸水力におよぼす外液のイ
オン価の影響

河原 昶：水草の葉の浸透価, Na 含量と透出に
ついて

高神 武・福田八十楠：植物組織の結合水測定方
法について

高神 武：植物の浸透価, 結合水および呼吸の水
分生理的相互関係

本田稔・福田八十楠：熱傾斜(temperature)と水
分傾斜(hydrature)との非相似性

福田八十楠：原形質膜の湿度制御機構

谷口森俊：相模湾沿岸の海藻群落

長谷川山雄：ミツイシコンブの生態学的研究(II)
生長と寿命とについて

口下部有信：池沼における水質の季節変化と
Trachelomonas 属藻類の消長

相馬寛吉：青森県東南部更新世泥炭の花粉分析と
植物叢生

堀田松雄：日本水質の環境的調査 II. 北アル
プス地帯

宗像和彦・鮫島惺一郎：北海道渡島・離島・小島の
植物相(予報)

北川昌典：九州中部山岳のマツ型森林の分布

高田幸之・小清水昭二・中谷 直：奈良若草山の
植物群落 2. ススキワラビ群落について

岩城英夫・佐伯敏郎・戸塚績：縞枯山森林の生態
調査報告(第 2 報)

小川房人・依田恭二：タイ国森林の概観

依田恭二・小川房人：タイ国モンスーン林の構造
と組成

森 千春：雑草遷移に関する一考察 雑草の放置
遷移の観察

清水正元：メヒシバ種子の休眠と外被

飯泉茂・菅原龟悦：放牧地における牛馬ふん上の
植物

田中瑞穂：ヒラシギスゲの根群

近衛廉也：ブロック法によるメタセコイアの根系
調査

植元 司：常緑広葉樹の葉および根の呼吸ならび
にその幼木の葉・茎・根の重量比について

高橋基生・渡辺匠美：環境変化(温度・水分その
他)と根系呼吸

- 高橋基生：越冬麦の生育経過と根系呼吸
生産速度と垂直分布
小林精・小谷信矢・田川日出夫・細川隆英：着生
植群の研究（2）着生植群と地形に基づく環境
細川隆英・小林精：着生植群の研究（3）着生植
群の構造動態
（炭酸石灰、置換性石灰および N/5 塩酸浸出 Ca
と植生との関係）
村田茂三：浸透圧の日変化と気象環境要素との関
連
生嶋 功：ウキクサの生長と栄養塩濃度との関
係
量の好適関係の定式化

展 示 講 演

- 藤原 勲：オウバコ属における倍数種の合成—六
倍種の合成
新崎盛敏：緑藻カサノリ目 Dasycladales 植物

シンポジウム

- トピック 1 亜種と変種について
10 月 26 日 9.00—12.00
北村 四郎：亜種と変種について
下斗米直昌：細胞遺伝学から見た亜種と変種
参加者約 140 名、原寛座長司会のもとに B 会場
で開かれた。
北村氏は、亜種と変種の区別が、形態
的特徴、また遺伝的、生態的、地理的、分布的
によつて、諸君の意見を求め、その結果として
は変種化による区別を認める。その結果、サブ
タクは規約上の一つのみ（3 名法）を認めるのかよ
いと述べた。これに関し北村四郎氏は同一種でも
分布がはなれること、鈴木時夫氏は分布が同じで
も別種とすべきものを例にあげた。また鈴

木氏が biotype の実在性について問うたのに対
し、下斗米氏はこれを支持し、鈴木氏は
これを支持した。次に北村四郎氏はフロラ研究の
歴史的経過から見て、始めは種を細分化し、後に
は統合する傾向の必然性を述べ、それを処理する
上で 3 名法の有用性を認め、過去における記載法
の短所について種以下のランクとしては変種より
亜種の方が確実性が高いことを述べた。これに関
し倉林、鈴木、長田、清水の諸氏から taxon
の実在性、type method についての批判、実
例や仮定による taxon の取扱ひ方についての
質問がなされたが、北村、木村、原の諸氏は命
題を指摘し、後者では複雑性を誰もが認めており、こ
れを前者に反映することで意見が分かると説
明された。鈴木氏の同じ group が人によって別
なランクに認められることがあるとの質問に、北
村氏はガンクピソウ類の例を挙げて、それが実際
に起こることを述べた。また鈴木氏によって、従
来は亜種と見られていたものが、最近では種と
指摘された。次に小林義雄氏は菌類では亜種、変種
は大して問題とならず、生化学的な strain, clone
が重要視され、特に下等菌類では形態が簡単なた
めに生理化学的性質が区別点となり、倍数性、分布
等は重要さが少ないことを例をあげて説明した。
そして strain や clone をどのランクにおくか
が問題となり、その結果として、種以下のランク
に置くことが述べられた。最後は下斗米直昌氏は、ノ
ジギク類、ミヤマヨメナ類、ワカサハマギク等を
例として倍数性と分布、系統の関係を述べ、学名
が重複するものがあることがあり、誤った印象をうけ易いことを指摘
した。これについて木村、北村氏から現行規約で
も系統関係を表わしうるように扱えることを、変
種、亜種、種を用いる場合の区別が、
た、木原均氏はコムギ属を例として核型、染色体
の対合、染色体の交換能力等の点から今まで 5 種
とされていたものが一種の中の亜種の関係におく
方がよいという結果になることを述べた。これで
4 人の話題提供を終ったが、すでに 12 時とな
り、本日はこの席を閉じた。

- トピック 2 核内要素の微細構造と化学
10 月 26 日 9.00—12.00

重永道夫：核および染色体の構造の電子顕微鏡的知見

太田敬久：紡錘体および隔膜形成体の細胞化学
水野忠款・山崎典子：Heterochromatin というもの

石田政弘：植物細胞核の DNA

参加者約 80 名。新家浪雄座長の司会によって C 会場で開かれた。

重永氏は根端組織のオスミウム固定、超薄切片法によって核および染色体の構造を観察し、1本の染色体は2本のクロモソームから、クロモソームは2本のクロモソーム II からというようにして、クロモソーム VI (10~20 m μ の太さ) までの階級、率からなることを報告した。また、隔膜形成体、細胞板などの電子顕微鏡的な構造をスライドによって示した。

つぎに太田氏は紡錘体と隔膜形成体の組織化学について報告した。これらにはタンパク、リピド、PNA が含まれるが、多糖類は含まれていない。しかし細胞板には多糖類は含まれている。また紡錘体は SH, SS に富む。核分裂後期の Interzonal region は特異な構造の部分で核分裂機構を解析するに重要な一つの手掛りになるであろうと述べた。

水野・山崎氏はヘテロクロマチンの研究発達史およびその定義を述べ、両氏がコアツモリで観察した結果を中心として総説した。

最後に石田氏は DNA の遺伝的に重要な役割をのべ、DNA の検出法としてのホイルゲン反応と細胞内要素や条件の変化との関係、ホイルゲン陰性と称せられる下等植物の DNA の性質などについて説明した。

以上の講演のあと、つぎのような総合討論が行なわれた。重永氏に対して、和田氏はオスミウムは紡錘体のよい固定液ではなく、むしろ CdCl₂ がそのせいいく構造をよく保持すると述べた。中村氏の染色体のマトリックスが見られないがという質問に対しては、オスミウム以外の固定液を考える必要を述べ、辰野氏はヘテロクロマチン部分の電顕的観察が行なわれるよう希望した。佐藤氏の細胞板形成の origin の質問に対し太田氏は隔膜形成体から形成されるらしいと解釈した。総合討論は主として核内要素の微細構造について集中された。

トピック 3 本質的な素代謝をめぐる諸問題

10 月 26 日 9.15—13.00

堀田康雄：ベニシダの前葉体初期における形態分化と核酸との関連について

太田行人・高田健三：発芽期植物における核たんぱくの存在様式

服部明彦：クロレラの窒素代謝について

まず堀田氏はベニシダの前葉体の生長が糸状体制の原糸体となる一次元成長と、それにつづいて平面状態体制になる二次元的成長とに分けられることに着目し、この成長形式、両者がアミク酸やアロウクで可逆的に阻害されることを示した。またこの阻害したたんぱく質は、核酸の量的および質的両面を追求し、この阻害による成長形式を規定しているものは RNA とたんぱく質の質的・量的変化であろうとした。

ついで太田氏は発芽期のミトリコサウの発芽時のたんぱく質および核たんぱくの行動についての研究の進捗状況を報告し、高田氏によつて、細胞の粒子構造に結びついた核たんぱくを電気泳動で分離し、その質的・量的変化を追求した実験結果を報告した。そして幼生組織では、ミクロゾームと結合して直接たんぱく合成に寄与する機能態 RNA と、構造からはなれて可溶性細胞質に移り、組織内を移動する移動態 RNA の二型があり、両者は相互に変化するという仮説を立てた。

最後に服部氏はクロレラ、*Chlorella vulgaris* の光合成と呼吸の形式を主題として述べたが、同時にこれらと関連する窒素代謝の中心についてのも議論が主眼結果が報告された。クロレラはウレアーゼ活性をほとんどないものと見做す、C₃ 固定から定量的に CO₂ を放出することから、尿素が何らかの形で一時的に結合し、これから CO₂ が放出され、残りは細胞質中にたまりまわるということを推定した。

このトピックの題は窒素代謝をめぐる諸問題というのであったが、実質上は二つの主題に分かれており、それぞれ示唆に富み、内容もかなり豊富なものであった。個別的な質問は、技術的な点から本質的なものにわたってかなりあったが、時間的制約のため討論にじゅうぶん時間がとれなかったことはまことに残念であった。もし問題点、あるいは討論を希望する点がいくつかあらかじめ設定されていたら能率的本質的に討議ができたかも

知れない。

(参加者 50—100 名)

トピック 4 植物群落をどのように考えて研究したらよいか 10 月 27 日 9.00—12.30

鈴木時夫: 標徴種による植物群落の研究

沼田 真: 群落統計の問題点

吉良竜夫: 実験群落による研究

門司正三: 生産構造について

総合討論においては、鈴木氏の活力度、沼田氏の優占度、種間の力関係という術語に関連して、術語とその定義の問題、群落の属性を表現する測度についての問題、さらに吉良氏の提起したレベルの問題の三つを中心に討論が進められた。

藤茂氏(岡山大)は生態学の術語が全般的に不明確で統一を欠いている点を指摘したが、高橋氏(東大)は、「活力度」という表現はとにかくとしてその内容について高く評価し、各方面からこの活力度を裏づけて行くことを提唱した。

測度の問題については佐々木氏(広島大)が活潑に発言し、被度の表現表について鈴木氏に質問したほか、沼田氏が優占度測定の測度として有効であると述べた *phytograph index* について、計算方法はまったく異質的なものを加えたり乗じたりしているが、その点は問題にならないかと質問したが、これに対し沼田氏は、異質的なものをなまのまま加えるのであればいけないが、相対値になおしてあるからかまわぬと答えた。

吉良氏は実験群落研究の意義について、各レベルにより法則性が異なるから、群落の法則は群落そのものを対象とする必要があること、実験に際しては各要因を *all combination* で追及すべきであることを強調した。これに対し、岩城氏(東大)から、自分等は器官レベルから群落の現象を解明しようとしている。器官→個体→群落と論理的につながりがつき、一段下の段階から順次説明可能ではないか。*all combination* とは非常に複雑なことになるが、具体的にどうかとの発言があり、吉良氏から、レベルのちがいを不当に強調しすぎたかも知れず、レベル間に断絶があるといったのは正しくない。しかし複合度の増大により現象にレベルの相違があることは明らかで充分認識して行かねばならない。先ほどの発言はむしろ操作的意味が強い。また *all combination* は誇張であ

るが、函数関係による予測性によって補いをつけようとしている旨、回答があった。

トピック 5 不和合性の問題

10 月 27 日 9.00—12.00

武丸恒雄: 菌類の不和合性因子

木村勘二: 菌類の不和合性現象に関与する変更因子

柴田寛三: アブラナおよびダイコン亜類の不和合性について

岡部作一: 顕花植物、とくにキク科における不和合性遺伝子の性質

(参加者約 50 名, C 会場)

田中信徳氏司会のもとに、まず武丸氏がエノキタケ NL-55 系統の実験を主にして、不和合性因子 A および B が、複対立性を有していることを強調され、これに対し及川氏は Buller 現象のよく起こる場合と比較して、複相菌糸形成の時の細胞学的所見を要求せられた。田中氏は限定二核化の際の培地中に生成される物質とか、培地の組成物質の作用というものに考及する妥当性を問われた。さらに同系統で示された比較的高頻度で起こる交叉について、これが同系統固有のものが、逆位等染色体異常によるものか、今後分析予定を述べられた。

ついで木村氏は、和合、不和合および両和合性の 3 型のものについて、Buller 現象は常に起こるが、両和合性の場合には特定組合わせの間にのみ和合菌糸が生じることについて、核の和合を調整する変更因子のはたらきを提唱された。及川氏は、以前行なわれた実験が、木村氏のそれによって明確化されたこと、田中氏は、同一子実体からの核の不和合性に対し変更因子よりも細胞質因子の概念導入を提案された。これに対し、同氏の結果からは、少なくとも細胞質の影響は考えられず、さらに変更因子については、分析結果に基づいて、一系列か二系列かきめねばならぬと述べられた。須藤氏および田中氏は、両和合性組合わせ $A^1B^1 \times (A^2B^2 + A^3B^3)$ と和合性組合わせ $A^1B^1 \times (A^2B^2 + A^1B^3)$ の場合とで、特定組合わせ和合菌糸の発生頻度が、 $A^1B^1 + A^3B^3$ が 78.3% に対し、 $A^2B^2 + A^1B^3$ が 37.3% と低下することをただされたが、これは、 A^1B^3 が A^2B^2 よりも早く $A \cdot B^1$ 菌糸の中を進むということと、 A^2B^2 , A^1B^3 間の和合性

の方が A^1B^1 , A^2B^2 間のそれよりも強いということで説明せられた。木原均氏は A^1B^1 および A^2B^2 の有する M^1 および M^2 の変異因子と A^3B^3 の M^3M^1 因子との間の組み換えによる分離比の変化をただされたが、やはり $A^1B^1 \times (A^2B^2 + A^3B^3)$ では $A^1B^1 + A^2B^2 : A^1B^1 + A^3B^3$ が 1:3 であるが、 $A^1B^1 \times (A^2B^2 + A^3B^3)$ では $A^1B^1 + A^2B^2 : A^1B^1 + A^3B^3$ が 5:3 となる。(岡山大学生物学紀要 4 巻, 1-2 号, 56-59 参照)。と後述された。最後に芳賀氏は Emerson 氏の行なった *Neurospora* のヘテロカリオン菌糸にみられる核の競走とか 増殖率につき、和合性菌糸のそれとの関係をただされた。次いで柴田寛三氏は *Cruciferae* の *Brassicinae* と *Raphaniae* の自家および交雑不和合性について 離反遺伝子説を中心に説明され、田中氏は *Salleles* の形質発現を変更させるような実験データを求められると同時に、Dr. Brewbaker (Brookhaven, National Laboratory) の premeiotic stage (corn) に対する X 線照射実験を紹介された。最後に岡部作一氏は顕花植物の場合として菊科にみられる不和合性因子の例証をあげ、興味ある解説を試みられた。

トピック 6 隠花植物における形態形成

10 月 27 日 9.00—12.0

中沢信午：フークス科藻類の実験形態学

新崎盛敏：カサノリ類 *Acetabularia* の体形成、とくに栄養枝、胞子枝の変動性について

猪野俊平・西林長朗：藻類の初期発生と器官発生との比較考察

野口 彰：センタイ類における胞子発芽と葉の再生

原田市太郎・伊藤道夫・菅井道三：モエジマシダの配偶体の形成、とくに細胞分化と相関関係について

参加者約 100 名。猪野俊平座長の司会の下に A 会場で開かれた。

まず中沢信午氏はフークス科藻類の卵が球形または卵形であって、その胚発生にあたってはまず一方の側に突起を生じてそこに仮根を形成することをとりあげて、極性軸決定の問題を述べられた。極性軸決定のメカニズムは皮部細胞質にあって、仮根形成部位にリビドの集積がみられる、な

どの説明から、一般に形態分化とは原形質の定向的增加である。換言すれば極性にほかならないと論じられた。新崎盛敏氏は緑藻の *Acetabularia* の栄養枝の形成の問題にはじまって単条の中軸の頂端にかさ状に輪生する胞子枝から配偶子が成熟するまでの形態形成上の諸問題を述べられ、この過程が環境条件の相違や外傷を与えることによっていろいろに変動することを指摘された。ついで猪野俊平氏は藻類の初期発生についてその発生型の下等なものと高等なものとの相違とその関係を総論的に解説され、西林長朗氏は藻類の後期発生の問題の中から褐藻類のコンブ目の単子嚢形成について、形態発生の進化を論じる目的からツルモ型、コンブ型、チガイソ型について説明された。以上の藻類における形態形成の問題につづいて野口彰氏はセンタイ類における形態形成の問題として胞子が発芽して原糸体を作るいろいろの形式について説明され、さらに原糸体の葉の細胞からの再生についてその難易を詳細に調査された結果を報告された。最後に原田市太郎、伊藤道夫の両氏は顕花植物とも共通の問題を多くもつシダ類の形態形成上の問題として配偶体の形成についての実験形態学的方法によった解析について詳細に述べられた。分裂域 原糸体部と中ろく部、翼部の細胞など、配偶体各部の間の相関関係ならびに再生能力について、また造卵器、造精器の形成について論じられた。

以上の諸問題が提出され討論が行なわれたが、藻類からシダ類までの非常にひろい範囲の対象についてその全般に共通した総合的討論を行なうことは至難であった。しかし各題目に対しての討論が行なわれて、中沢信午氏は伊藤道夫氏に仮根形成と造精器形成との関係について質問をされたが、伊藤氏はその直接的関係を否定された。

今回のシンポジウムでは極性の問題、栄養部分と生殖部分との形成上の関係、生殖器官の形成など形態形成の根本にふれる問題が中心となったが、その議論を展開すべき時間の不足が痛感された。

話題の選択とその範囲、参加人数、時間など、今後さらにシンポジウムを有益なものに育てるためによりいっそうの努力が望まれるが、シンポジウム形式が学会にもちこまれてから間もない現在、シンポジウムそのものの意義をじゅうぶんに

確認することができたものと思う。

懇 親 会

10月27日総会終了後、3台のバスを連ねて今宵の懇親会場、博多帝国ホテルへ向かう。

生花が美しく飾られたテーブル、床にはガーデンさえしつらえられ、三日間の難しい議論に明暮れた面々も、おのづと心もなごみ、インドのサーニ夫人や服部会長夫人および小島大会会長夫人をはじめ大会出席者同伴の御夫人達も交えてカクテルパーティは進められていった。150名余の出席者は旧交を温めあう人達あるいは懐古談に花を咲かす額縁大会名誉会長および同夫人等の長老組、そしてまたグラスを片手に、昼の講演会場から続きの Discussion をする若い人達と、あちこちにできたさまざまなグループからなごやかな談笑の音がホール一杯にひるがって、さしもの広い会場も狭さを感じられるほどの盛会さである。最初は懸念されないでもなかったこの初の試みのパーティ様式もスムーズに進行し、親睦を求め、話題を携えて行きかう人々や、あるいは腹ごしらえよろしく料理をパクつく人達等、いずれも心楽しい雰囲気醸成しながら学会最終日の宵もふけて、8時すぎ、それぞれ懇親の実をあげ、会はなごやかな談笑のうちにその幕を閉じた。

エクスカーショ

△福岡市内および近郊見学班 (Aコース)

10月28日8時20分三大八幡宮の一つ宮崎八幡宮前に集合、参加者35名。西鉄バス配車手違いにより約1時間20分遅れて出発、バスガイドの説明に耳を傾けながら一路紫筑路を太宰府に向かう。遅れを取り戻すため、途中の都府楼跡、観世音寺は車を止め、車中より説明を聞くのみで太宰府天満宮に向かう。10時20分天満宮着、約30分間壮大な境内を心ゆくまで観賞、「東風吹かば……」で思い出される「飛び梅」に菅原道真の当時を偲びながら久留米の九州農試園芸部に向かう。園芸部では50分間にわたる説明見学後昼食。13時20分、A B A Cコースと分かれふたたび福岡市に向かう。市内、黒田五十二万石を今に伝える舞鶴城跡を左に見ながら、その濠外からなる水の公園、大濠公園をバスで一周、最後の目的地西公

園に14時40分着。博多湾に面した頂上展望台より、博多湾、玄海に文永、弘安の役の昔を偲んだ後、終着博多駅前に向かう。15時40分無事駅前で解散する。

△阿蘇・別府方面旅行班 (Bコース)

阿蘇・別府方面旅行班は28日午後博多駅を出発、途中久留米駅でA Bコース班と合流する。総勢31人。熊本で豊肥線に乗りかえ阿蘇の内之牧「ひのくに荘」に午後7時に着く。晩秋の月明りの下に阿蘇中岳の火柱を遠望する。翌朝8時半に「ひのくに荘」を出発。10時過ぎ山頂の火口壁にたどり着く。遠雷のごとく轟き、等身大の石を盛んに噴き上げている。草千里で足下の「りんどう」をめでながら中食をとる。12時半に下山、途中造り物のような「こめつか」の山に歓声を上げ、猪野教授の名ガイドぶりにしばし耳を傾けける。坊中で国鉄に乗りかえ一路別府へ。鈴木時夫教授の出迎えで直ちに別府「豊泉荘」に向かう。大分市長および鈴木教授から銘酒の寄贈があり夕食の座は大いに賑わう。それぞれお国自慢がとびだす。翌朝9時に「豊泉荘」を後にし温泉熱研究所に向かう。宮沢所長の熱心な説明に耳を傾け珍らしい熱帯植物に目をみはる。10時過ぎ同所を辞し高崎山に向かう。400匹余のオサルの出迎えを受け、一同御満悦。最後に大分市長の待つジャングル公園へ車を走らせる。係員が会員一同から植物の種名や栽培法等についていろいろ意見をうけたまわる。ふたたび車で引き返し午後1時半、別府棧橋および別府駅前で無事解散する。

△雲仙・長崎方面旅行班 (Cコース)

この班は二手に分かれ、一つは福岡から直接大牟田に向かうもので、もう一つは途中バスで都府楼跡、観世音寺、太宰府天満宮、九州農事試験場園芸部を見学するコースであった。出発の28日は晴天に恵まれたが、後のコースはバス会社の手落で出発が1時間20分も遅れた。太宰府天満宮では息子達の受験が成功するようにお守りを買う会員もいた。園芸部ではガーベラが赤かった。久留米で福岡から直接大牟田に向うコースと一緒に。不知火海上の船ではほとんどが上甲板で近づく雲仙峰をカメラにおさめる。29日、硫黄臭い温泉、吹き出す水蒸気、その上に載せてある卵、喬木・灌木の紅葉にまじるアカマツの緑、黄色のゴルフ場、妙見岳より見下した光る海、両側の車窓

に均等に関ける眺望。下山のバスには肥満型を山側に坐らせる慣行になっている由。かくて車は近代日本植物学の始祖ゆかりの地長崎へ。30 日晴天。海を見下すグラバー邸には花にも秋は深かった。崇福寺と中国服を着た老人の拝観料徴収係。出島のツェンペリー記念植物。シーボルト邸跡。諏訪公園にあるツェンペリー記念碑の前に設立委員長長崎大学の外山三郎先生に立ってもらい、一同シャッターを切る。長崎駅にて仙台での再会を約し解散。

△被同伴者市内見学班

服部会長夫人に伴なわれたサーニ夫人および大会出席会員の夫人等総勢 19 名、細川教授夫人の案内で 10 月 27 日午前 10 時に会場受付前を出発し新宮のマルティ・グラス工場見学に向かう。すばらしい色彩の製品や巧みな工作過程に一同眼を見はる。ついでバスガイドの説明を聞きつつ宮崎宮・東公園と廻り、西公園で昼食、午後大濠公園を通して松居博多織工場に向かう。全操作過程を見学、とくに手織工の優れた技術に皆驚く。ついで博多人形師高尾八十二氏の工作所を見学、ここでも全過程を説明してもらう。午後 4 時ここで解散する。御婦人達だけに博多織、博多人形については活潑な質問があり、有意義な見学旅行だった。

△第 2 回名古屋対東京定野球試合

前回の東京大回から始まった東京大学と名古屋大学の野球の試合は、今年も大会の第 1 日と第 3 日の 2 日間にわたっておこなわれた。両軍の打撃大いに振い、結局 22-20 の驚異的なスコアで、去年につづいて名大の 2 連勝におわり、江戸の仇は長崎で討てず、来年の仙台にまで持ち越されることになった。

近 畿 支 部 例 会

昭和 33 年度第 1 回例会 (7 月 12 日、於京大・理・植物)

清水建美：石灰岩地帯の植物群集の問題

藤田安二：精油成分より見たるトウバナ属の分類と系統

桃谷好英：*Brassica Rapa* L. における種子たん白の個体変異について

田村道夫：キンボウゲ科における葉柄の維管束 [特別講演]

広瀬弘幸：日本産温泉植物の研究

なお今年度の近畿支部の役員は次のように決定いたしました。

支 部 長 芦田 譲治

庶務幹事 日下部有信 (京大・理・植)

〃 広野 好彦 (京大・農・応植)

会計幹事 横村 英一 (京大・理・植)